SPLEEN PUNCTURE

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BY

SVEN MOESCHLIN

Privatdozent University Medical Clinic Zürich

Translated by

A. PINEY, M.D.



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(3) Puncture must not be performed in the presence of a recent septic splenomegaly or in the case of a painful spleen whether due to stretching of the capsule or to infarction

(4) Unconscious patients must never be punctured

(5) Rigid aseptic precautions must be taken

We are convinced that in time spleen puncture will become much more widely used because it is of such great value in the diagnosis of splenomegalies of clinically uncertain origin In addition this method gives valuable information as to the condition of the spleen in various diseases and as a result of different therapeutic methods Puncture of the sternal marrow, combined with puncture of glands and of the spleen often throws considerable light on the course and distribution of various diseases of the hæmopoietic system

The purpose of the present book is the discussion of the technique and the results of spleen puncture based on some 300 personal observations between the years 1939 and 1949 in the Zurich Clinic in the hope that this method will obtain a much wider acceptance At the same time the literature of the subject is critically discussed and correlated with personal observations

CONTENTS

PART I GENERAL

1 HISTORICAL

SPLEEN puncture was probably first performed by Vidal and his school for the diagnosis of typhoid fever towards the end of the last century when the bacilli were obtained in culture For the same purpose Hayashihava performed 109 punctures (1905) without any complications Later spleen puncture was performed in the Mediterranean countries for the diagnosis of Leishmaniasis (Kala Azar) The procedure was used for the same purpose in France by Nicolle (1909) Aravantinos (1916) Coronia (1922) Giraud (1925) Benhamou (1934) and in Spain by Pittaluga (1935) The first clinical publication from Central Europe was that of Nagy (1924) to whom the credit should be given for introducing the method of puncture during deep inspiration He pointed out that all the accidents previously observed (Wohlgemuth) were due to laceration of the capsule when spleen puncture was performed during expiration or in a mid way position because contraction of the diaphragm during puncture produced movement of the spleen in relation to the fixed needle Nagy stresses the value of this method of examination for clinical diagnosis Mele (1925) reached the same conclusion on the basis of 55 cases in Algiers In pædiatrics Nassau (1926) employed spleen puncture for diagnosis and in one case of miliary tuberculosis was able to discover tubercle bacillus in films

In Italy spleen puncture was used mainly for the diagnosis of Hodgkin s disease Introzzi (1932) was probably the first to describe the typical Sternberg cells in films from spleen puncture—a finding which was later confirmed by Storti (1935) Gosio (1947) has carefully investigated the spleen in malaria Spleen puncture was also used quite early for the diagnosis of Gaucher s disease (for literature see p 198) All the publications so far mentioned have dealt with individual diseases and Weil (1934) together with Isch Wall and Perles were the first to undertake systematic investigation of the various forms of splenomegaly by spleen puncture In Poland Tempka (1938) attempted to investigate the normal spleen by puncture of the organ during laparotomy More recently Forconi (1939) has reported in detail on spleen puncture in erythroblastosis and there is a Spanish publication by Lopez (1939) dealing with splenomegaly in general Heilmeyer and Schoner (1941) have also described the condition of the spleen in erythroblastosis Spleen puncture in mononucleosis was described by us in 1941

In the more recent text books on hæmatology spleen puncture does not receive much attention in fact only Heilmeyer (1942) lays

much stress on its diagnostic value and reports on his own observations in Hodgkin s disease erythroblastosis and Gaucher s disease while Ferrata and Storti (1946) also attribute great value to it

It is undoubtedly, going too far to suggest that spleen puncture is of greater diagnostic value than sternal puncture—a view which Weil supports indeed, he writes as follows ' Donc si a premiere vue les resultats (des myelogrammes) semblent satisfaisants une experience plus etendue montre que les classifications adoptees pour la rate n ont pas ici (c est a dire dans la ponction sternale) la purete et la precision que nous observons dans les splenogrammes '

We certainly cannot agree with this view but it is undoubtedly a mistake to undervalue the significance of spleen puncture for diagnosis and we are convinced that in time this procedure will come to occupy a valued place in clinical investigation

2 TECHNIQUE

ESTIMATION OF THE SIZE OF THE SPLEEN

(a) Palpation and Screening

It has already been mentioned that only definitely enlarged spleens should be punctured in most cases it suffices to rely on palpation of the organ If however the size of the spleen can only be recognised by percussion one should not attempt puncture unless very distinct enlargement is demonstrable In all doubtful cases before deciding on spleen puncture it is advisable to examine the patient with the fluorescent screen when as a rule the organ can be seen lying between the bubble of gas in the stomach and the diaphragm where it appears as a dark oval shadow If this is not demonstrable but if percussion has really aroused a suspicion that the spleen is enlarged one can try the following method while the patient is being examined with the fluorescent screen he can be given 10 grammes of bicarbonate in a little water followed by a small amount of lemon squash the gaseous distension of the stomach will then make the spleen easily visible

(b) Adrenalin Test

In some cases further assistance in deciding whether a swelling in the left hypochondrium is really the spleen can be obtained by an intramuscular injection of 1 mg of adrenalin If within 15 or 20 minutes there is a distinct diminution in the area of mass there is then no doubt that this consists of the spleen (Gosio *et al*) A negative adrenalin test is however not conclusive evidence that the mass is not composed of the spleen but it is well worth while examining the blood 15 or 30 minutes after the injection because even if no contraction of the spleen can be detected by palpation there is

often an emigration of immature cells from this organ (for literature see Hortling)

It is essential to realise that the presence of movement on respiration is not conclusive evidence that a mass consists of the spleen because even a hypernephroma or a pancreatic cyst may move in a similar manner

(c) X ray

An enlarged spleen is usually detectable on an ordinary chest film several workers have however attempted to make the organ more easily visible by injection of radio opaque substances which are stored in the reticulo endothelium At first Thorotrast was tried but was given up because its radio activity was dangerous Jentzer and Weyeneth (1944) have used Hepatoselectan which is a colloidal preparation of iodine which enables the spleen to be visualised very distinctly Gosio (1947) and others have carried out similar investigations

The use of such radio opaque substances is only of practical value when it is necessary to decide whether a palpable area of resistance in the left hypochondrium is really a spleen or whether it is due to some other tumour The fact that Hepatoselectan is stored only in the liver and spleen not in such masses as hypernephroma cysts sarcoma etc gives definite information In the majority of cases however percussion palpation and screening amply suffice

SITE OF PUNCTURE

Most authors have chosen the site for puncture in the area of the greatest definite dullness on percussion whether this happened to lie medial to the costal arch which involved puncture through the abdominal wall or laterally inserting the needle through the lowest intercostal space For the sake of safety we have limited ourselves to puncture the spleen in the ninth or tenth intercostal space a little more laterally or medially according to the position of the diaphragm on deep inspiration and according to the size of the area of splenic dullness Weil also seems to prefer this procedure

The topography of the spleen Fig 1 shows that puncture of the organ at the level of the ninth or tenth intercostal space in the position of complete inspiration will avoid all the adjacent organs The colon which lies antero laterally cannot be reached at this level and the stomach is also safeguarded by its medial position and by the interposition of the spleen The lower lobe of the left lung may extend down into the phrenico costal angle at the level of the tenth intercostal space but puncture of this is quite harmless because of the absence of any large vessels in the peripheral part of the lung Such penetration of the lung can usually be avoided if the lowest limit of pulmonary resonance during full inspiration is determined

by light percussion then the needle can be inserted 5 cm *below* this level When the puncture is performed more in the medial part of the ninth or tenth intercostal spaces the lung is avoided because the lobe of the lung does not reach so far medially

We regard puncture of the spleen through the abdominal wall as being definitely contra indicated even when palpation seems definitely to exclude the interposition of some other organ *e g* the omentum stomach, etc , between the abdominal wall and the spleen There is no doubt that it is extremely difficult to be certain of this

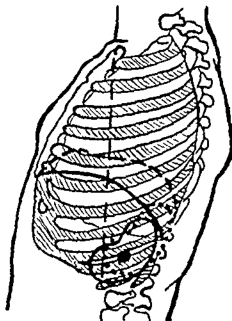


FIG 1 The broken line shows the position of the spleen in expiration and the continuous line that in inspiration *Puncture is performed in the ninth or tenth intercostal space in the mid axillary line according to the area of maximum dullness and three or four fingers breadth from the costal margin the whole procedure being carried out while the breath is held after a full inspiration*

especially in obese patients Furthermore direct puncture of the spleen through the abdominal wall does not permit of such certain fixation of the needle which may be displaced by contraction of the abdominal muscles and so cause small laceration of the splenic capsule Also direct puncture because it is done more medially and the spleen lies deeper increases the chance of confusion so that a greatly enlarged left lobe of the liver or a hypernephroma or a pancreatic cyst can be misleading This is not likely to occur when careful percussion in the lateral region at the level of the ninth or tenth intercostal space fails to elicit dullness But even using the intercostal route such surprises may occur and we ourselves

have inadvertently punctured a hypernephroma and also a pancreatic cyst. The main argument in favour of the intercostal method is the fact that the upper pole of every definitely enlarged spleen lies in the left lateral sub-diaphragmatic space directly in contact with the ribs without the possible interposition of any other organ.

THE PUNCTURE NEEDLE

Weil used a needle 0.8–1 mm. wide and 10 cm. long with a stylet and a 2 or 5 c.c. record syringe. In our experience a rather wider and longer needle 1.2–2 mm. in diameter and 12–15 cm. in length with a ground-in stylet is better. This increases the chance of aspirating small fragments of spleen tissue and the length of the needle usually suffices to prevent any of the material from entering

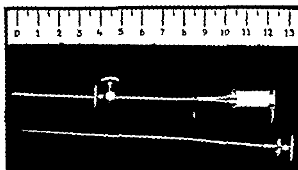


Fig 3. Puncture needle with stylet and movable guard

the syringe—an event which usually spoils the result because the cells are disintegrated by being blown out of the syringe again.

The bevel of the needle should not be steep but it should be extremely sharp because most of the material is obtained during its insertion. If no special needle is available an ordinary lumbar puncture needle will serve. In all cases there should be a movable guard on the needle to prevent too deep a penetration. Both the needle and syringe must be sterilised and dry because even the smallest amount of water disturbs the normal cell structure.

The syringe should be a very well fitting 20 c.c. record syringe with which powerful aspiration is possible. Smaller syringes such as were used by Storti and Weil are not so satisfactory because of the lesser degree of negative pressure.

Spleen Puncture by the Harpoon Method

It seemed obvious that spleen puncture with a liver puncture needle of Roholm and Iversen or with Silvermann's needle might be tried so as to obtain definite pieces of spleen suitable for histo

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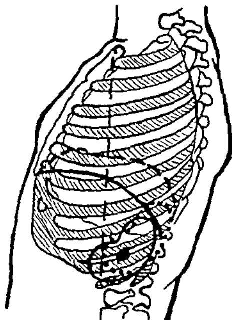


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and a 5 cm record syringe containing 4 c c of a local anæsthetic e g 2 per cent procaine It is recommended that no risk should be taken with the sterility of the anæsthetic and that only ampoules should be used and not solutions sterilised in bottles because these are liable to contamination when used several times First a cutaneous wheal should be made with the local anæsthetic and then the tissue should be infiltrated perpendicularly to the skin surface to a depth of 1–2 cm the patient is then asked to breath rapidly and superficially while the needle is slowly pushed on a millimetre at a time until there is a complaint of slight pain which usually indicates that the peritoneum has been reached The needle is then pushed on 1 or 2 mm further until one can feel a scratching sensation as the point rubs against the spleen capsule this depth should then be marked on the needle If as is occasionally the case such vibration cannot be felt the depth of penetration to be marked on the needle is that at which the slight peritoneal pain was noticed this is practically the same distance as the surface of the spleen *This method permits exact measuring of the depth of puncture required to reach the splenic capsule* Thus if we add 2 cm to this depth it enables us to fix at the same time the depth of the puncture in the spleen tissue itself and to limit it to 2 cm

This modification of the technique of spleen puncture which we were the first to describe *permits a limitation of the penetration of the spleen to a minimum and thus prevents damage to any of the larger vessels* The main vessels of the spleen enter at the hilum and end as a fine mesh work in the sub capsular region on the lateral aspect *i e* the site that is punctured In this way there is no danger of penetrating the larger vessels at the hilum even if the spleen is not greatly enlarged and is of the flat type Slow and careful introduction of the needle used for local anæsthesia rarely fails to demonstrate the distance of the spleen from the surface If however as a result of extensive adhesions and a large almost immobile spleen it is not possible to determine the depth one must carefully penetrate to a depth of not more than 5–7 cm according to the degree of adiposity

Having anæsthetised the area and decided on the exact depth of penetration the spleen puncture itself can be commenced The guard on the needle should be fixed to 1 5–2 cm beyond the depth of the capsule and the needle should be pushed through the same spot between the ribs then the stylet is removed and the record syringe attached The patient is now told to take a deep breath and then to close his mouth and pinch his nose between two fingers while the spleen is rapidly punctured As soon as the guard prevents further penetration one aspirates quickly but strongly once or twice Before the needle together with the attached syringe is withdrawn it is essential to release the negative pressure in the syringe by per

logical examination We would however warn anyone against the attempt because by this method of tearing a cellular piece of tissue out of the spleen far too many blood vessels are opened and as the needle punctures more deeply, there is the possibility of opening large vessels and so leading to severe bleeding In any case the examination of such larger pieces of tissue seems to be unnecessary in the majority of cases because it is particularly in the spleen that the structural characters of the cells and their quantitative distribution are best seen in films which certainly permit finer detail to be seen than do histological sections in which the cells are shrunken by fixation This applies particularly to the difference between lympho sarcoma and lymphatic leukæmia, etc In the liver the conditions are very different because the histological structure rather than the cellular composition is the important feature If however the histology of the spleen is to be investigated it is best to use a needle of 3 mm in thickness and not to penetrate the organ more than 1 cm The technique of histological examination of such fragments is described on p 10

Technique of Puncture

Before undertaking a puncture, the bleeding time (Duke s method) and the coagulation time (Lee White s method) should be estimated the former being more important If this is considerably prolonged puncture should not be performed If the bleeding time *is normal but the coagulation time slightly prolonged, puncture can* usually be performed without danger If both values are increased it is better to avoid performing a puncture Slight diminution of platelets and of prothrombin are usually of no significance if the bleeding time is normal

Before proceeding with the puncture one must obtain a clear picture of the position of the spleen using most careful percussion and palpation The patient should lie flat on his back with no pillows under the head Puncture of the spleen with the patient in the lateral position is not to be recommended because the spleen then falls towards the middle line One should first percuss the spleen with the diaphragm in the middle position and then tell the patient to take a deep breath The exact upper limit of dullness should be marked on the skin and if light percussion is used this corresponds with the lower limit of the lung The puncture should be made at least 6 or 7 cm below this point in an area of complete dullness almost invariably this point lies 4 or 5 cm lateral to the costal margin in the mid axillary line in the ninth or tenth intercostal space

The area is carefully disinfected with merthiolate or iodine either of which can be used for indicating the point of puncture by a cross For local anæsthesia we use a fine needle about 10 cm long

Negative Punctures

When the area of splenic dullness is definitely increased puncture rarely fails to obtain some material If however the organ is very hard as after intensive X ray treatment for Hodgkin s disease nothing may enter the needle

If in spite of dullness two punctures prove abortive X ray screening should be done to prove that the spleen really is enlarged If it is a puncture a little to one side of the original one may be successful

After treatment and Possible Complications

Weil keeps his patients lying on their left side with an ice bag on the hypochondrium for 12 hours As the puncture is so super ficial when our technique is used we do not employ these precau tions We have been able to ascertain that punctures performed 1 or 2 days before death cannot be recognised at autopsy and that there is no trace of blood in the peritoneum

Spleen fluid clots more rapidly than blood probably because of the very numerous platelets in the pulp and the liberation of thrombokinase from the damaged tissue cells And as our puncture only extends into the sub capsular tissue there is probably reflex contraction of the vessels which in this region are all small For this reason we do not advocate post operative injection of adrenalin

As Nagy and Weil emphasise the few published examples of bleeding from spleen punctures were probably due to laceration of the capsule by puncturing the organ in the position of expiration or by puncturing a soft septic spleen or by failing to recognise the presence of a hæmorrhagic disease

The irritation of the puncture may cause a reflex contraction of the diaphragm and if the operation is not performed during full inspiration the spleen may be lacerated by movement against the needle Hence the method here advocated should be carefully adhered to and in more than 300 spleen punctures we have never seen any signs of bleeding

After the operation the patient should lie flat on his back for an hour but no ice bag is needed He can then have a meal and remain in bed for a further 6 hours The only complaint that may be made is of a slight stitch on inspiration which may be referred to the left shoulder We then use a Dilaudid suppository and have always found that there is no further trouble Out of 300 patients 8 com plained of slight stitch lasting for 2 or 3 days but in all other cases only very slight discomfort was mentioned The operation itself should be completely painless

With proper precautions infection does not occur If however a spleen abscess is suspected puncture should only be performed extraperitoneally during a laparotomy

mitting the plunger to go back slowly If this is not done negative pressure may bring blood or local anæsthetic into the syringe which makes the films useless For the same reason no further aspiration must be performed while the needle is being withdrawn The patient must hold his breath in the inspiratory position during the whole procedure and may only breathe again when the needle has been withdrawn It is essential to pay particular attention to this and to practice the whole procedure apart from the puncture itself with the patient before the actual performance of the operation

The inspiratory position is a basic requirement for the harmlessness of spleen puncture and for this reason the operation must not be performed on unconscious patients

If puncture is performed properly no blood enters the syringe but a few drops of serous fluid and perhaps a few bits of tissue can be blown out of the needle In splenic inflammation and in cases in which there is portal hypertension or chronic congestion of the spleen only brief aspiration should be performed for fear of drawing blood into the syringe The smaller the amount of blood sucked out the more cells there will be found in the films and they will be in a better state of preservation so that a more accurate splenogram can be obtained

Gosio recommends that no aspiration should be performed and that one should limit one s examination to the material that enters the needle during the puncture Our own impression is that short aspiration does however supply more material—particularly from firm spleens

Preparation of Films

The material must be spread rapidly because spleen fluid coagulates even more quickly than marrow from sternal puncture As soon as we have withdrawn the needle it is taken off the syringe, which is filled with air and re attached to the needle then the contents are blown out on to one or more slides excess of blood can usually be removed with filter paper but this should not be necessary with good technique Then with the edge of a suitable slide films are made of one or two drops of the puncture fluid Of course if only one or two drops are obtained it is as well not to try to make more than one film The spreading must be done without pressure and requires a certain amount of practice which can be obtained by making films from gland or sternal puncture

If there are some fragments of tissue in the needle the histological method suggested by Rohr for sternal marrow can be used viz fixation and embedding in paraffin (see p 10) obviously this combined cytological and histological method has much to recommend it

states small collections of pigmented cells may be recognisable These appearances are not constant and are much less important than the microscopic ones

As a rule one can dispense with a detailed qualitative determination of the splenogram Those given in this book are mainly intended as standards of comparison for other workers

A detailed differential count is useful in some conditions such as aleukæmic lymphadenosis in which an increased percentage of lymphocytes is diagnostic The splenogram is also useful in some cases of chronic myeloid leukæmia and cases of osteosclerotic and myelofibrotic anæmia The qualitative relationships of the various cells and the degree of maturity are often of prognostic significance in leukæmia Apart from this the detailed splenogram is mainly of academic interest

Low magnification (× 300) should be used for a preliminary examination but in order to avoid glare it should be covered with a thin layer of cedar oil At least two films should be thoroughly examined using the oil immersion lens for any peculiar cells The best parts of the films should be fully examined with the highest magnifications It is only in this way that scanty elements such as the Sternberg cells of Hodgkin s disease are likely to be detected

The aspirated spleen tissue is derived from the red pulp as well as from the follicles so that the different types of cells are not evenly distributed in the films The cells from the pulp are found more evenly spread than those from the follicles which tend to occur in streaks (Fig 25) Certainly marrow and gland films spread more evenly than does spleen juice Of course experience helps a great deal For instance if there is much blood spleen cells will be more widely distributed but will be found mostly at the edges of the films This is a sound reason for not trying to obtain too much spleen juice one or two quick aspirations suffice to obtain enough material in the needle Even so a good deal of blood may be obtained then it may take time to ascertain whether any spleen material is present If pulp cells or macrophages are seen there can be no doubt of the success of the puncture

Marking of Cells

It is often useful to mark the position of cells so that they can be found again The following is a simple method of doing this A film is firmly clipped on the movable stage and the desired spot is examined with the oil immersion objective This is then raised and the oil on the film is carefully removed but a small amount is left on the aperture of the objective The tube is now racked down until the objective touches the preparation A small ring of oil will be seen when the objective is raised and the spot can be marked on the back of the slide by a small ink circle

For anatomy and physiology of the spleen refer to the literature on the subject

A young man developed signs of generalised sepsis after a panaritium with great splenomegaly but repeated blood cultures were negative and chemotherapy proved useless A spleen abscess was suspected and an operation was decided on The spleen was surrounded by dense adhesions and puncture revealed staphylococcal pus Recovery followed drainage of the splenic abscess

Recent infarction is a contra indication to spleen puncture (Stubenrauch) because the friable tissue easily tears Spleens that are tender on palpation must also not be punctured because the tenderness is clear evidence that the capsule is tense

Staining Methods

Air dried films are stained by the May Grunwald Giemsa method allowing the Giemsa solution to act for 15–20 minutes instead of the usual 10 minutes A few films should be retained for special staining *e g* Sudan if lipoid storage cells are present or Prussian blue staining if iron containing pigments are suspected (hæmo siderosis etc)

Histological Methods

If enough tissue is obtained it is advisable to embed one or more fragments for histological examination We use the method suggested for sternal marrow by Rohr

Fix in sublimated formol (saturated solution of sublimate 400 40 per cent formalin 100 glacial acetic acid 20) for 1 to 2 hours

Wash in running water 1–2 hours
70 per cent alcohol 12–24 hours or longer
96 per cent alcohol about 12 hours
Change of 96 per cent alcohol 12 hours
Absolute alcohol 2 3 hours
Chloroform ½–1 hour

If the material is bloody use Carnoy s solution (absolute alcohol 6 chloroform 3 glacial acetic acid 1) instead of 96 per cent alcohol

Embed oven at 60 C —soft paraffin (46°–48) 2–3 hours, then hard paraffin (56) 2–3 hours Cool in water and block

Sections 2–3 microns thick are mounted and placed in the oven (60) for 1–2 hours After cooling 10 minutes in Xylol then Xylol and alcohol equal parts followed by absolute alcohol I and II and then 70 per cent alcohol Wash in water remove sublimate by placing in Lugol s iodine for 10 minutes then wash for ½–3 hours Stain with hæmatoxylin and eosin or Romanowsky

Examination of Films

As a rule little can be learned from the naked eye appearances but in leukæmic and lymphosarcomatous spleens the puncture fluid may be greyish and mixed with little blood In hæmolytic

plasmacytoid reticulum cells and lymphatic plasma or Turk cells The *purely morphological differences* between these two groups which will be discussed later are probably not alone sufficient to justify the subdivision but the great functional difference supports such a distinction

Rohr and many other investigators (Nordensson Schilling Heilmeyer etc) believe that the plasma cells found in the blood arise from the plasmacytoid reticulum cells of the bone marrow and that they are therefore not as was previously believed derived from the lymphatic system (Maximow Turk Pappenheim Naegeli) Clinically there is an increase of plasma cells in the blood in a variety of diseases especially in German measles and in infective hepatitis We have undertaken a systematic investigation of the relationship of these elements in the sternal marrow and in gland puncture of cases of German measles and have been able to demonstrate that the plasma cells found in the blood certainly are not formed in the bone marrow but in the enlarged lymphatic glands (Moeschlin 2) In gland punctures of these cases one can find every transition from immature cells probably derived from the reticulo endothelium to typical young and also mature plasma cells such as are found in the blood (Fig 21) whereas in the marrow there is no increase of plasmacytoid reticulum cells or of the younger elements of the plasma cell series We have called the parent of the plasma cells in lymphatic glands the plasmoblast (Fig 11 *a* 21 *a*) which is quite different from the lymphoblast This special type of cell only occurs in lymphatic tissue and our investigation suggests that it is probably directly derived from the large lymphatic reticulum cell which occurs in the germ centres

These plasmoblasts are distinctly seen in histological preparations and the presence of such large cells with large vesicular nuclei was observed in the glands in cases of German measles by earlier investigators (Debenedetti) These plasmoblasts with their large delicate nuclei are not found in the myeloid marrow even when there is considerable hyperplasia of the plasmacytoid reticulum cells *e g* in pneumonia Even so our view has been controverted by some writers (Dubois Ferriere Heilmeyer etc) who hold to the opinion that the plasmacytoid reticulum cells and the plasma cells are morphologically and functionally one On the contrary Landolt carried out an investigation on infective hepatitis and by sternal puncture was able to show that the plasma cells found in the blood were not formed in the marrow confirming our own investigations Our own opinion based on spleen puncture in hepatitis (described later) was that the lymphatic tissue of that organ must be regarded as the site of formation of the plasma cells Ludin who performed gland punctures agrees with the above view and Gormsen also recognises two separate series of plasma cells

3 CYTOGENETIC PROBLEMS

Our classification of blood cells follows that of Naegeli who *regarded the myeloblast as being the differentiated stem cell of the* granulocytes We have not differentiated reticulo endothelial cells into hæmohistioblasts fibroblasts etc agreeing with Rohr that bias as to their genetic and functional state must be avoided It is best to name the various reticulo endothelial elements on purely structural grounds

Naegeli s views are opposed by the Italian school (of Ferrata) with whom Kienle has joined forces

Ferrata derives the erythropoietic granulopoietic and thrombo poietic series from the hæmocytoblast which is structurally identical with the non granular myeloblast of Naegeli while the Italian school refer to those cells that contain fine azurophile granules (promyelo cytes of Naegeli) as myeloblasts They use the term hæmohistio blast to refer to a cell that to some extent at least corresponds with *the parent of all blood cells and which in German literature is* usually known as the Ferrata cell It is probably no longer advisable to use this term because some of the elements known as hæmo histioblasts are non granular while others contain some granules and can therefore scarcely be given the status of stem cells Naegeli *and Rohr have pointed out that many of these so called Ferrata* cells are artefacts and are really crushed premyelocytes or damaged leukæmic cells The illustrations of more recent Italian hæmato logists (Fieschi and Storti) indicate that the non granular forms are identical with the lymphoid reticulum cells of Rohr while the pictures of granular hæmohistioblasts given by Kienle are by no means convincing On the whole therefore we adhere to the views of Naegeli admitting however that the inferences as to cyto genesis are scientifically vulnerable and will doubtless require modification in future Certainly our views of the genesis of mono cytes and plasma cells differ from earlier opinions

Monocytes

Naegeli regarded the monocytes as being purely of myeloid origin whereas Schilling held that they are of endothelial origin *Probably the truth lies midway between these views and we share* Rohr s opinion that there are both myeloid and lymphatic monocytes (*e g* in some cases of lymphatic reaction) and also that some arise directly from the reticulo endothelium as in some infective states (reticular monocytes histiomonocytes) (see Fig 16 III *k*) We have *been unable to find any support for Wuhrmann s view that the* monocytes play a part in the formation of proteins

Plasma Cells

On the basis of earlier investigations we have already asserted that the plasmacytoid elements can be divided into two groups viz

and in the marrow but not in lymphatic tissue Such new experimental results appear to confirm the view that there are functionally two types of plasma cells

Investigations with the phase contrast microscope (Moeschlin 23 24) showed that the cytoplasm of myeloma cells contained rather large dark granules which are not identical with the mitochondria and which do not stain with ordinary staining solutions (Fig 20 *e*) It is possible that these structures are really pathological high molecular globulins which are formed in these cells It is interesting to note that we found similar dark globular structures in those active plasmacytoid reticulum cells (Fig 20 *d*) in inflammatory conditions (? anti body formation) and also in small lymphoid reticulum cells (Fig 30 *g*) Fagraeus appears to have shown conclusively that the latter can be directly converted into the plasma cytoid type Other pathological conditions in which there is a great increase of such small reticulum cells associated with striking changes in the blood proteins have also been recognised *e g* macroglobulinæmia of Waldenstrom On the contrary the lymphatic plasma cells of German measles showed no dark granules *in the examination by phase contrast* (Fig 20 *b c*) At the present time our interpretation of these findings is to be regarded as nothing more than a working hypothesis which we hope to be able to prove by further observations

We used to believe that the extramedullary myelomas which are perhaps partly derived from the lymphatic plasma cells do not *produce any changes in the serum proteins (Jaeger) This is not* confirmed by the latest investigations (Jequier Doge Heckner 1949)

Another differential feature is the possession of phagocytic powers by the plasmacytoid reticulum cells a function which is not manifested by the plasma cells of the blood The plasmacytoid reticulum cells do not only ingest fats and lipoids (see Fig 16 *a*) (Gormsen Fagraeus etc) but also hæmosiderin We have seen this in cases of hæmolytic anæmia and also of aplastic anæmia in which repeated transfusions have been given (see Fig 17 *d*)

Dubois Ferriere (1943) demonstrated this phagocytic activity by injection of Indian ink into the marrow We could however never detect any signs of phagocytic activity on the part of the plasmoblasts or of the blood plasma cells which we regard as being of lymphatic origin We cannot agree to the opinion of Dubois Ferriere that the plasma cells are a state of lymphocytes or reticular cells characterised by the absorption of proteins Especially the interesting examinations of Fagraeus who compared the number and the morphology of the plasma cells in the spleen with the titre of the anti bodies in the blood contradict this opinion

Plasmacytoid reticulum cells with typical reddish violet cytoplasm and relatively small nucleus are found in the spleen as are

In addition these investigations of the bone marrow and the lymphatic glands show that there are functional differences between the two series of cells The plasmacytoid reticulum cells of the marrow appear to play a part in the formation or metabolism of certain globulins (Brass Rohr Fleischhacker, etc) This certainly applies to the β and γ globulins which are found in myelomatosis and which may pass through the kidney (Bence Jones proteoses in the urine) and also into the cerebro spinal fluid (increased total protein abnormal mastic and gold sol reactions) (see Moeschlin (9))

The electrophoretic investigations of Wuhrmann and Wunderly etc show that the abnormal proteins produced by the myeloma cells mainly belong to the group of γ globulins and are much more rarely of the β or α group It has, therefore been inferred that the plasmacytoid reticulum cells of the marrow which are the parent cells of the pathological myeloma cells are the source of at least a part of the serum globulin (Rohr Fleischhacker Klima etc) Rohr especially called attention to the parallel increase in the number of plasmacytoid reticulum cells and the level of globulin fraction in the blood in various diseases *e g* chronic inflammatory diseases and cirrhosis of the liver

Gormsen (1942) confirmed that this was approximately true while more recently he has been able to show that these plasmacytoid reticulum cells probably possess the function of producing anti bodies as was first suggested by Huebschmann (1913) Dougherty and White attributed this function to the lymphocytes (see p 43) but Gormsen has shown in animal experiments, that the thymus which is so rich in lymphocytes does not form any anti bodies while extracts from the spleen (which contains many plasma cells) and also of the peri renal fat (which contains no lymphocytes but many plasma cells) contain much greater concentrations of anti bodies than do other bodies or the blood serum (Bjorneboe Gormsen and Lundquist (1947)) Bing Fagraeus and Thorell (1945) using the micro spectrographic method of Caspersson showed that this synthesis of anti bodies as well as the synthesis of cell protein occurs in association with ribose nucleotides Fagraeus (1948) was able to extend Gormsen s work in so far as she found the highest concentration of anti bodies in the spleen of immunised animals when immature plasma cells were most numerous Such anti bodies were very scanty in the lymph follicles of the spleen but plentiful in the red pulp Kolouch (1947) observed that in sensitised rabbits the plasmacytoid reticulum cells decreased in size in the state of shock and changed into the small Marschalko type within four days

These investigations justify the conclusion that the plasma cytoid reticulum cells play a great part in the formation of anti bodies and this process occurs mainly in the red pulp of the spleen

increase of cells characteristic of infective reactions or the presence of pathognomonic elements such as epithelioid or Langhans giant cells or glandular fever cells etc In leukæmias and sarcomas films may contain a few characteristic cells or these may be so numerous as to dominate the picture

The discussion will be divided into that of the cells found in the normal spleen and that of genuinely pathological forms but the latter will be dealt with later when individual diseases are discussed

NORMAL SPLEEN CELLS

Strictly it is not true that the films are made from normal spleens because puncture is only done when there is distinct enlargement Our knowledge of the normal spleen cells is therefore based on spleens removed at operation

The cells can be divided into the following five groups —

A Reticulo endothelial elements
B Cells of the lymphatic series
C Immature myeloid cells from hæmopoietic foci
D Mature granulocytes
E Thrombocytes

A RETICULO ENDOTHELIAL ELEMENTS

Aschoff used the term reticulo-endothelium to designate the totality of cells that are characterised by the power of forming reticular fibrils and of picking up colloidal dyes after injection In the spleen the reticulo endothelial apparatus is composed of the framework of the red pulp and the endothelial cells of the sinuses together with those cells that form the mesh like stroma and the lymph sinuses of the follicles The latter is identical with the reticulo endothelial cells of lymphatic glands and for this reason spleen films will be found to contain those elements that are characteristic of the red pulp as well as those of the types found in gland punctures

The nature of the germ-centre cells as Thiel and Downey call them is still disputed although transitions between them and lymphoblasts and large lymphocytes certainly do occur We have therefore included them with the lymphatic cells although we are *convinced of their reticulo endothelial nature and have used the* name large lymphatic reticulum cells

Forkner (1928) was one of the earliest to subdivide the reticulo endothelial cells into four groups — reticulum cells fibroblasts macrophages and tissue mast cells Pavlovsky (1934) only recognises endothelial cells and macrophages Tischendorf (1939) in his monograph on gland puncture speaks of undifferentiated endothelial reticulum cells fibroblasts macrophages and plasmacytoid reticulum cells while Stahel recognises young round cells sinus endothelium cells macrophages and tissue mast cells Rohr was the first to subdivide and describe the

also the rather larger lymphatic plasma cells with a relatively looser and larger nucleus As far as the available facts are valid it seems that plasmacytoid reticulum cells are present and are formed in the red pulp The red pulp of the spleen thus plays a part in infective diseases by producing certain anti bodies and some globulins of the γ group whereas numerous lymphatic plasma cells are formed in the follicles (white pulp) whence they pass into the blood stream

Ectopic Foci of Blood formation in the Spleen

It has long been held (Naegeli) that in adult life the spleen has lost its embryonic power of producing erythroblasts and myelocytes Spleen puncture has shown that erythroblasts are rarely found in the normal spleen of adults but that a few erythroblasts and myelocytes are present in all infective splenomegalies and sometimes in larger numbers in hæmolytic anæmias It seems clear that the spleen can regain erythropoietic powers although this can be of no great sig nificance in health because the number of erythroblasts is small On the other hand distinct foci of formation of red cells and granular leucocytes develop in diseases in which the medullary spaces of the bones are decreased in size, as in osteosclerosis and some tumour like states when there is usually a considerable degree of splenomegaly

The existence of scattered granulocytes and even of myeloid foci in adult spleens has been known to pathologists for many years Sternberg (1906) mentions them as being a variable but normal constituent of the spleen pulp and these observations were con firmed in the spleens of cases of sudden death and of executed criminals (Weidenreich 1911 Weill P 1920 and Bertelsen 1938) Hartmann also agrees that a few immature granulocytes are normally present in adults and careful examination of films from our own series of spleen punctures has revealed a few such elements in almost every case The number is small in slight infec tions but greater in septic conditions

These observations together with the evidence of tissue culture and the knowledge that marrow tissue may be found in areas of secondary calcification (e g in the lungs lit see Lang) show that in certain conditions of post natal life the undifferentiated mesen chymal cells can again give rise to the manifold types of blood cells This subject is further discussed in the sections on splenomegalies

4 CELLULAR STRUCTURE OF SPLEEN FLUID

The cells seen in films from spleen puncture are partly blood cells and partly derived from the splenic tissue itself As a rule the majority are lymphatic elements with some cells from the reticulo endothelium and perhaps some from hæmopoietic foci Infections alter the composition so considerably that the films may show an

PLATE I A NORMAL RETICULO-ENDOTHELIAL CELLS IN SPLEEN PUNCTURE

(from a water colour picture by Mrs Bollinger Schudel)

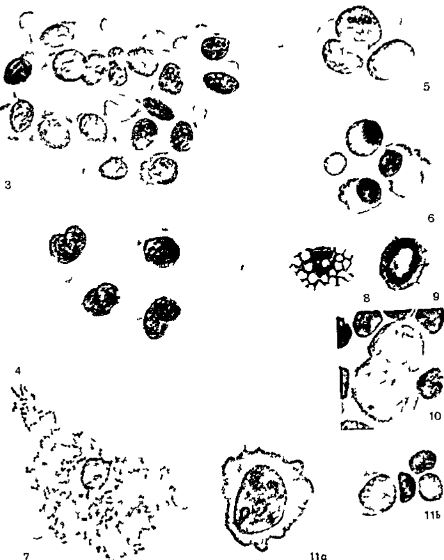


FIG 3	Serosa cell syncytium	FIG 9	Tissue mast cell
FIG 4	Pulp cell syncytium	FIG 10	Large lymphatic reticulum cells (germ centre cells)
FIG 5	Separate serosa cells	FIG 11a	Lymphatic plasmoblast
FIG 6	Plasmacytoid reticulum cells	FIG 11b	Lymphoblast and three old lymphocytes
FIG 7	Large blue pigment macrophage		
FIG 8	Lipophage		

[F f p pag 1

various reticulum cells of the marrow and his four groups are generally accepted He distinguishes macrophages lymphoid reticulum cells plasmacytoid reticulum cells and true endothelial cells

Our own observations on gland puncture (Moeschlin 4) have led us to the following classification — (1) large lymphatic reticulum cells (2) foreign body and pigment macrophages (3) fat and lipoid storage cells (lipophages) (4) lymphatic plasmoblasts (5) tissue mast cells This classification has been adapted to fit the lymphatic reticulo endothelial elements in the spleen by adding the cells peculiar to that organ viz, serosa and pulp cells as well as those common to it and the marrow—capillary endothelium and plasma cytoid reticulum cells We have attempted as has Rohr a grouping on purely morphological grounds ignoring functional subdivision into histiocytes fibroblasts and fibrocytes Rohr distinguishes the large lymphoid reticulum cells with immature loose nuclei from the small lymphoid reticulum cells with small dense nuclei It seems likely that the larger forms with their immature loosely constructed nuclei correspond at least in part to the fibroblasts while the smaller ones are comparable with the older elements—fibrocytes and resting cells of the marrow reticulum

The lymphoid reticulum cells possess the ability to develop into erythroblasts or even abnormal cells such as megaloblasts and epithelioid cells Certainly *in vivo* studies of marrow (Nordensson Rohr Tischendorf *et al*) and gland puncture (Tischendorf, Stahel) confirm the occurrence of these changes

We subdivide the reticulo endothelial cells found in spleen punctures as follows —

(1) Serosa cells
(2) Pulp cells
(3) Small lymphoid reticulum cells
(4) Macrophages (*a*) cell debris macrophages
(*b*) pigment
(*c*) bacteria
(*d*) lipophages (fat and lipoid macrophages)
(5) Tissue mast cells
(6) Plasmacytoid reticulum cells

1 SEROSA CELLS

Like other intra abdominal organs the spleen is covered by a single peritoneal layer of endothelium the so called serous coat As spleen puncture involves only a small area of the serosa very few of the cells are found in films, but unlike other elements they are usually seen in small groups If ascites is present a small amount of the fluid may enter the needle and then a few isolated serosal cells sometimes showing degenerative changes may be seen as in films of ordinary ascitic fluid

Structure The cells are large (20–40 μ in diameter) and are usually found in groups (Figs 3 5 12 *a*) Their characteristic structure distinguishes them from all other cells although there may be a slight resemblance to those of the pulp which however have greyer cytoplasm

The cell is large and round without a distinct cell membrane and contains a round or oval nucleus which is often eccentric Its size varies from 12–24 μ and its basichromatin is rather coarse and close sometimes

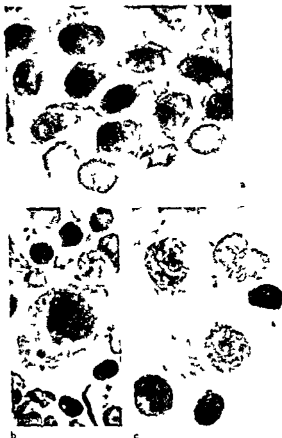


FIG 12

(*a*) Serosal syncytium from spleen puncture
(*b c*) *Pulp cells characteristic of spleen puncture (b) Shows a single* cell containing ingested pigment (*c*) A group of incompletely separated cells

with 1 or 2 nucleoli 3 μ in size These are seen as pale areas which stain bluish in the younger forms The cytoplasm stains pale blue and often contains evenly scattered fine azurophile granules We found these cells only 11 times in 170 spleen punctures (see photomicrographs in Fig 12 *a* and coloured picture 3)

2 PULP CELLS

These cells demand attention because they have not previously been described during life Lubarsch mentioned certain elements

them from the tissue fragments They are most numerous towards the end of the film often in a sort of reticular distribution of small but dense collections That such elements are not scanty in the tissue itself is clear from histological sections which are seen to contain many small almost naked nuclei very like erythroblasts and small lymphocytes For this reason these cells cannot be included in the qualitative splenogram

The cells are small with almost naked round or slightly oval nuclei The chromatin is almost homogeneously arranged in a solid mass in which nucleoli can be detected in the most immature forms As said

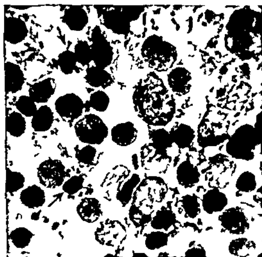


Fig 13 Small type of reticulum cells scattered among normal lymphocytes Frequently it is not possible to distinguish these elements from old lymphocytes

above the cell can often not be distinguished from old small lymphocytes seen in spleen smears

Similar cells are found in gland punctures They are probably slightly differentiated mesenchymal cells which can develop into macrophages or fibroblasts It is also possible that such stimuli as for instance inflammation may cause their conversion into lymphoblasts erythroblasts and perhaps myeloblasts thus explaining the genesis of ectopic foci of hæmopoiesis

In spleen films we have not been able to recognise the large lymphoid reticulum cells that Rohr described in marrow films but this must not be confused with our large lymphatic reticulum cell as seen in spleen films (Figs 10 19) These latter are found in both spleen and gland films probably corresponding with those from the germ centres hence our inclusion of them in the lymphatic series

found in post mortem smears which may be identical with the cells here described Probably the elements we term pulp cells correspond with those known to histologists as spleen sinus cells or as sinus endothelium These cells are found in all films from the spleen and their presence in films is proof that the material is derived from that organ Neither in the marrow nor in lymphatic glands are identical cells found and we consider them to be the only morphological element peculiar to the spleen although their specificity has not previously been recognised because they were often supposed to be monocytes e g by Weil Once their exact structure has been recognised the differences between them and other cells presents no difficulty

In normal spleen films they form 0 2–0 6 per cent of the cells but rise as high as 7 per cent in inflammatory conditions

They are often seen as indistinctly bounded elements with irregular edges to the cytoplasm probably because in health they form a firm cellular tissue becoming free only in abnormal conditions e g infections Occasionally they are found in connected groups of 3 or 4 cells (Fig 12 c) but the majority lie isolated among other types of cells

Structure The cell body is usually elongated reaching a length of 40–50 μ sometimes with a tail like projection at the ends The nucleus (15–20 μ) usually lies eccentrically and is sometimes slightly indented or even almost lobulated thus somewhat resembling younger monocytes The finely granular but closely packed chromatin which stains bluish violet only possesses nucleoli while it is still in the immature basophilic stage

The cytoplasm is grey unlike that of the serosal cells sometimes with patches of reddish violet and usually contains fine granules varying from reddish violet to grey (see Fig 4 on Plate I and b in Fig 12) In a few darker and coarser granules are seen probably representing ingested ferruginous pigment Our view is that these elements probably develop into typical macrophages certainly an uninterrupted series of transitional forms can be recognised

3 LYMPHOID RETICULUM CELLS

As already mentioned the framework of the marrow glands and spleen is made up of histologically undifferentiated reticulum (mesenchymal) cells which are also known as fibroblasts and fibrocytes Rohr distinguishes two types large and small The latter (small lymphoid reticulum cells) are undoubtedly present in spleen films although they cannot always be distinguished from old or atrophied lymphocytes It is only when the large lymphocytic type predominates (Fig 13) or these cells lie among the myelocytes in cases of myeloid leukaemia that they are recognisable with certainty

As in the case of marrow films the cells that form the supporting tissue are scanty or crushed because of the difficulty of separating

occur in glands liver marrow and spleen Hæmosiderin macrophages in the marrow have been described by many writers

The *hæmosiderin storage cell* (Fig 14 *e*) varies in size from 20 to 50 μ The nucleus is small and usually oval lying eccentrically The whole of

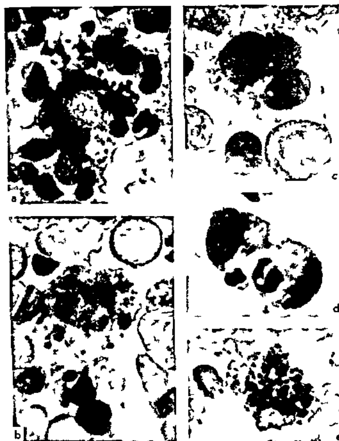


Fig 14 Cell-debris and pigment macrophages

(*a* and *b*) Large macrophages loaded with nuclear remnants and pigment
(*c*) Macrophage with 3 nuclei from a case of splenomegalic cirrhosis
(*d*) Macrophage with ingested leucocytes The pyknosis in the latter indicates that there has been phagocytosis and that the appearance is not an artefact
(*e*) Hæmosiderin macrophage from a case of chronic hæmolysis

the cytoplasm is closely packed with rather coarse often slightly angular masses of dark brown or yellowish green pigment which do not usually invade the nucleus No sharp cell outline can be found in most cases because the cells have been torn out of their reticular connections For the same reason portions of protoplasm filled with masses of pigment but containing no nucleus are common The cytoplasm is almost colourless and often invisible

4 MACROPHAGES

Probably all reticulum and possibly endothelial cells are facultative macrophages Certainly, in spleen films every stage intermediate between small reticulum cells and pulp cells on the one hand and macrophages on the other can be recognised It has been supposed that the plasmacytoid reticulum cells possessed no phagocytic powers but Dubois Ferriere has shown experimentally that particles of carbon are found in them after injection of Indian ink And in sternal marrow Rohr has observed transitions from these cells to lipophages In aplastic anæmia with early hæmosiderosis due to repeated transfusions we have seen iron pigment in these elements

The macrophages can be subdivided into four groups according to the type of ingested material although mixed types do occur —

(*a*) *Cell debris macrophages*
(*b*) Pigment ,
(*c*) Bacteria ,
(*d*) Fat and lipoid (Lipophages)

In sections, macrophages are seen in both the red pulp and the lymphatic nodules of the spleen Those that contain cell debris are identical with similar elements in the marrow and glands but, in the spleen, certain pigment and fat macrophages of a type not found in those organs may be seen

(*a*) *Cell debris Macrophages* Such cells are also found in gland punctures (Forkner Tischendorf Stahel Moeschlin (4) Stuyt Strunge) and in marrow films (Rohr *et al*) They are a constant feature of spleen films (0 1–0 2 per cent) increasing to 0 8 per cent in infective conditions

Every kind of transition from smaller forms (20 μ) to giant ones of 60 μ can be found The ingested material is usually nuclear or cytoplasmic detritus but may consist of a whole cell (Fig 14 *a*) while an occasional cell is seen to contain carbon or to be encrusted with calcium

Only cells showing signs of degeneration (nuclear pyknosis cytoplasmic vacuolation diffuseness of outline etc) are ingested and we believe that the published pictures of whole cells inside megakaryocytes represent artefacts

The structure of the nuclei of these macrophages resembles that of the lymphoid reticulum cells but in the older forms is extremely dense In the young types the rather loosely arranged nucleus is central and may contain a small blue nucleolus while in old forms it is more elongated and lies at the edge of the cell (Fig 14 *a b*)

(*b*) *Pigment Macrophages* This group includes all those cells that have picked up break down products of hæmoglobin or other pigments There are two main groups viz those with iron containing and those with iron free pigment both of which are well known to

Gosio found cells laden with very dark melanin in spleen puncture (see Fig 33 *b c*) This pigment is quite different from the brownish or greenish yellow tint of hæmosiderin and its presence is a valuable sign of malaria even many years earlier

(*c*) *Bacteria Macrophages* Schilling and more recently Rohr noted the occurrence of endothelial cells and of monocyte like elements of reticular origin in the blood in infective states (endo carditis) Ingested bacteria may be seen in the cytoplasm for instance we have seen a reticulum cell containing morphologically recognisable pneumococci in a spleen puncture performed during severe pneumonia (Fig 15 *b*) We have also seen a chain of strepto cocci in a monocyte (Fig 15 *c*) The discovery of such bacterial phagocytosis supports the view that the spleen plays an important defensive part in transient and more chronic bacteriæmias thus being of importance in both humoral or cellular immunity

Parasite Macrophages For the parasites of malaria phagocytosed in reticulum cells (see Gosio) and of Kala Azar (see Fig 33 *d*) Refer to the chapters on these diseases in the second part

(*d*) *Fat and Lipoid Macrophages* (*Lipophages*) Fat and lipoid storage cells are scanty in the spleen (1–2 in every 10 000 cells) in one case of sepsis lenta we found eight such cells in a whole film We have previously mentioned their occurrence in gland punctures so that it seems probable that those in the spleen are derived from its lymphatic follicles *i e* from its reticulum cells Their presence in the spleen has long been known (Lubarsch Kusunoki) but they have not previously been recognised in films In sternal marrow Rohr showed that both small reticulum cells and plasmacytoid reticulum cells could gradually become large fat cells and in spleen films it is possible to find every kind of transitional form First the fat globules in the cytoplasm are small and scanty but ultimately these elements become converted into large fat cells (Fig 16 *a–d*)

The nucleus of the large cells lies near the edge of the cells as is the case in the marrow A few macrophages however stain faintly and contain closely packed small vacuoles in the cytoplasm prob ably indicative of lipoids

In the larger elements the nucleus is usually central (Fig 16 *e f*) These are probably identical with the cells we described in gland punctures (large lipophages)

Large Monocytoid Macrophages We found these elements (Fig 16 III *i* and *k*) most often in chronically inflamed spleens and Gosio has described and illustrated similar cells from the spleen in chronic malaria They store pigments such as hæmosiderin and melanin as well as lipoids Their origin is probably from various reticulo endothelial elements

They are quite large cells with indistinct edges The nucleus is slightly indented and the chromatin although fine is densely arranged usually

Blue Pigment Macrophages In a case of splenomegaly in a man aged 29 of uncertain but not hæmolytic nature we found macrophages which contained closely packed fine granules which stained deep azure blue (Fig 15 *a* and Plate I Fig 7) Such blue pigment macrophages have not previously been described in gland or marrow punctures but Dr Brausil, of Zagreb has sent us a marrow film in which identical cells together with large lipophages



FIG 15

(*a*) Large so-called blue pigment macrophage These cells are filled with the pigment that stains azure blue with May Grunwald (see Fig 7)
(*b*) Bacteria macrophages
(*c*) Monocyte with chain of cocci from endocarditis lenta

and many plasmacytoid reticulum cells were present The specimen was from a case of suspected chronic malaria

If such blue masses escape from crushed cells they may be mistaken for masses of bacteria or precipitates of stain Unlike the hæmosiderin cells these elements usually have a round central nucleus which often contains a definite nucleolus

The blue stained granules are very fine and may be so closely packed that the whole cell body appears blue then the granules can only be recognised at the edges The nucleus is usually free from granules The cells vary from 30–60 μ

The nature of the pigment is unknown but we suspect that it is an iron free derivative of hæmoglobin

Malaria Melanin Macrophages In cases of chronic malaria



FIG 16

III

(*l*) *Large monocytoid macrophages* in a chronic inflammatory spleen The monocytoid nucleus with cytoplasm containing fat and other ingested materials is characteristic of these cells which somewhat resemble Gaucher cells (× 1 700)

(*k*) *Histiocytic monocytes* from the spleen with denser arrangement of chromatin than in myeloid monocytes These cells are particularly numerous in chronic inflammatory spleens (× 1 000)

with single pale areas The cytoplasm resembles that of Gaucher cells and stains pale reddish violet with small cloud like areas of pallor in contact with one another (Fig 16 III *i* and *k*)

5 TISSUE MAST CELLS

These cells are scanty (1 in about 10 000 cells) but are increased in chronic inflammations *e g* we saw 0 1 per cent in sepsis lenta and 0 2 per cent in lymphatic leukæmia

Rohr (1948) observed increase in the marrow in aplastic anæmia and also in a peculiar case of chronic interstitial inflammation of the marrow

They are not to be confused with the basophiles of the blood (Weil Strunge *et al*) which are essentially different from the mast cells of the spleen marrow and glands There are a number of distinctive differences that indicate their genetic and functional disparity The basophiles of the blood develop from basophile myelo

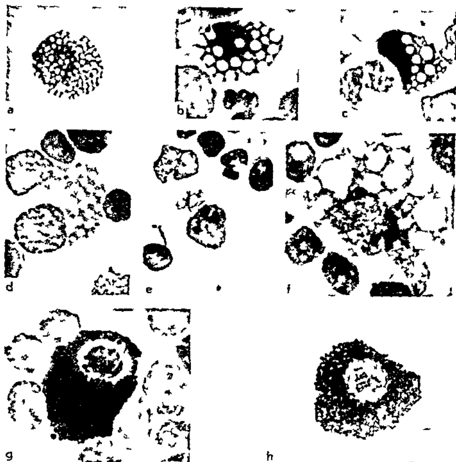


FIG 16

I Fat and lipoid macrophages

(*a*) Plasmacytoid reticulum cells showing early phagocytosis of fat
(*b* *d*) Typical fat cells
(*e* and *f*) Large lipophages with central nuclei similar to those common in glands but scanty in marrow

II Tissue mast cells

(*g* and *h*) Note the fine granules that do not involve the nucleus and compare with blood basophiles with their larger granules and narrower cytoplasm

The nucleus is usually eccentric and relative to the cytoplasm is small It is rounded and in older specimens shows a cart wheel distribution Only the younger cells show the presence of a small single nucleolus Multi nucleate forms are not uncommon in chronic inflammation (Fig 17 *b* and *c*) Some contain fat globules and so represent transitions between the plasmacytoid reticulum cells and the large lipophages They may also pick up hæmosiderin (Fig 17 *d*) and as Dubois Ferriere has shown particles of injected Indian ink

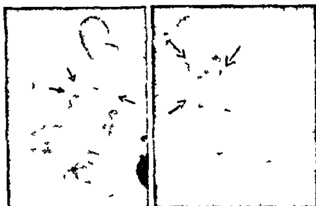


Fig 17 *d* Phagocytosis of hæmosiderin in plasmacytoid reticulum cell

The distribution curve of the sizes of these cells is the same in spleen and marrow films (Comparative measurement each 50 cells in marrow and spleen) This fact together with the morphological evidence demonstrates their identity (Fig 18)

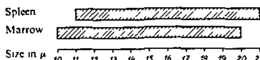


Fig 18 Variations in size of plasmacytoid reticulum cells

Structural Differences between the Plasmacytoid Reticulum Cell and the Lymphatic Tissue Cell

The reasons for distinguishing two types of plasma cell have already been discussed (see p 13) but their morphological differences deserve consideration

The plasmacytoid reticulum cell (Fig 6) has a more violet blue cytoplasm and in the younger ones is narrower than in lymphatic plasma cells The nuclei of the youngest stages of the former type are smaller and more compact with coarser chromatin than the delicate structure of the young lymphatic plasma cell (plasmoblast) The latter shows 3 5 distinct pale blue nucleoli which persist into

cytes *i e* they belong to the myeloblast series whereas the tissue mast cells which never appear in the blood, arise locally in various tissues arising directly from reticulum cells Pappenheim Maximow, Naegeli *et al* showed that they do not give the oxydase or indo phenol blue reactions as do the hæmic basophiles Possibly the tissue mast cells play a part in regulating the coagulability of the blood by producing heparin as shown by Holmgren and Hilander (1937) and confirmed by Jorpes (1947) and Paff Bloon and Reilly (1947) Ehrlich and co workers (1949) positively proved the pro duction of heparin in these cells

Tissue mast cells (14-25 μ) possess a redder finer and more closely packed granulation than do hæmic basophiles The cytoplasm is fairly wide and the granules lie round the nucleus (Figs 9 16 *g h*) whereas in the basophile leucocytes the coarser and darker granules are scattered over the lobulated nucleus

6 PLASMACYTOID RETICULUM CELLS

The plasmacytoid reticulum cells seen in spleen films are identical with those found in the marrow Their numbers vary from 0 1 to 0 3 per cent in normal conditions to 10 per cent or even 17 per



FIG 17
(*a*) Plasmacytoid reticulum cells of the same type as those of the marrow (*b* and *c*) Two large multi nucleated cells in which mitosis was not followed by division of the cytoplasm

cent in chronic inflammatory conditions They are also increased in hepatic cirrhosis

In films and sections they are usually found in groups of 2 5 cells For discussion of their functions and for the findings in the phase contrast see pp 12 16 and Fig 20 *c d e*

Structure In the spleen the size varies from 11 to 21 μ The cyto plasm is usually rather more violet blue than the azure blue cytoplasm of the larger lymphatic plasma cells and is often riddled with vacuoles but less often contains azure granules The edge is indistinct and rough

as seen in sections especially because of their characteristically vesicular nuclei their identity must be admitted

Some writers call these large vesicular cells of the germ-centres "macrolymphoblasts" (Hartmann) while others regard them as being reticulo endothelial (see Bloom) We are convinced that study of gland and spleen punctures clearly show transitions from these elements to lymphoblasts and plasmoblasts *i e* they are the parents of the hæmic plasma cells while they can also be converted into glandular fever cells (lymphoid monoblasts) The large lymphatic reticulum cells of the germ centres are thus to be regarded at least in part as the reticulo endothelial stem cells of the lymphocytic series

In normal circumstances lymphocytes regenerate from lymphoblasts but in conditions of stress the large lymphatic reticulum cells from the interior of the germ centres probably become active and give rise to lymphoblasts and plasmoblasts These *lymphatic* cells differ from the large *lymphoid* reticulum cells of the marrow in the presence of much looser chromatin in their large nuclei with very distinct large nucleoli They differ from lymphatic plasmoblasts by their central nuclei with finer masses of chromatin enclosed in a narrow zone of cytoplasm whereas they can be distinguished from lymphoblasts by their size although transitional forms are also found

2 LYMPHOBLASTS

Normally only 0 1–0 2 per cent are found in spleen films and it is surprising how few are found in most lymphatic leukæmias (see p 122)

These cells are 11–18 μ in diameter The pale blue cytoplasm is rather narrow and the nucleus has a fine but close network of chromatin in which 1–2 distinct nucleoli are visible (Fig 11 *b*) The main distinction from the larger myeloblasts lies in the greater density of their chromatin

3 LYMPHOCYTES

(*a*) *Young Small Lymphocytes* These are fairly small cells with narrow cytoplasm and compact nuclei in which 1 or 2 pale areas or even nucleoli can be seen Normally they form 0 6 7 5 per cent of the total cells

(*b*) *Mature Small Lymphocytes* These elements have dense nuclei with no trace of nucleoli

(*c*) *Young Large Lymphocytes* In this group (0 4–3 per cent) are included all the larger cells with wide cytoplasm (13–18 μ) and either a nucleolus or a circumscribed pale area in the nuclei

(*d*) *Mature Large Lymphocytes* These are the elements (2–5 per cent) with rather wide cytoplasm (11–17 μ) and no distinct pale area in the nuclei

a fairly mature stage while even the least mature examples of the plasmacytoid reticulum cell have only one small nucleolus

Even when mature the lymphatic plasma cell retains the pachychromatic structure of the nucleus which lies more eccentrically than that of the plasmacytoid reticulum cell Even the mitoses differ (see p 38 and Fig 23 *a b c d*)

Phagocytosis of lipoids and hæmosiderin (Fig 17, *d*) may be present in the plasmacytoid reticulum cells but is unknown in lymphatic plasma cells

B LYMPHATIC SERIES

1 LARGE LYMPHATIC RETICULUM CELLS

In punctures of inflamed glands we found a few elements that we call large lymphatic reticulum cells (Figs 11 *a* 19) These are large (20–25 μ) with narrow dark blue cytoplasm and indistinct outline, while the large round or oval nucleus contains 3–5 nucleoli in its delicate chromatin As far as can be ascertained from pictures published by others these are identical with the hæmocytoblasts found in gland puncture by Pavlowsky the undifferentiated endothelial reticulum cells of Tischendorf and the young round cells of Stahel



Fig 19 Large lymphatic reticulum cells (germ-centre cells) with a narrow dark blue cytoplasm and a large nucleus These cells can be found in spleen films and in gland punctures and are identical with the germ-centre cells

A few of these can be found in spleen punctures and where a follicle has been penetrated and spread out it is clear that these cells belong to the lymphatic tissue of the spleen They form 0–0 1 per cent of the cells of the normal spleen but may rise as high as 1 per cent in lymphatic reactions

Their structure indicates that they are only slightly differentiated young cells which probably arise from the reticulo endothelium of lymphadenoid tissue they are not found in marrow films Our studies on gland puncture and spleen puncture seem to us to show that *these elements are the constituents of the germ centres of the lymphatic follicle* i e *are identical with the germ centre cells* described by Thiel and Downey in histological sections Certainly their number is increased whenever germ centres are numerous As no other cell found in films could be identical with these elements

Structure The plasmoblast is a fairly large cell (17–30 μ) of rounded or slightly oval shape with a central or eccentric large round nucleus (12–25 μ) The cytoplasm stains cornflower blue with May Grunwald often with a pale area on one side of the nucleus especially in the more mature forms (Figs 11 *a* 20 *a*)

In the young forms the cytoplasm is bulky and the cell membrane



Fig 21 Development of lymphatic plasma cells based on gland puncture in German measles (See *Helv et Med Acta* 7 ? 7) An identical series can be detected in spleen films

so delicate that it is easily injured and hence apparently separated pieces of cytoplasm or cells with torn edges are often seen Some have vacuoles in the cytoplasm similar to those seen in mature hæmic plasma cells

We have never found any granules whether with May Grunwald Janus green pyrodin or the peroxidase method or the phase contrast microscope but the cytoplasm of some of the deeply basophilic cells is distinctly cloudy

Such subdivision of the lymphocytes is convenient in differential counting but transitional forms are quite common

4 PLASMOBLASTS

The origin and nature of these cells has already been discussed (pp 12–16)



FIG 20 *a* Large young lymphatic plasma cells (*Plasmoblast*) with typ al eccentric nucleus and large cornflower blue cytoplasm in spleen film of epidemic hepatitis

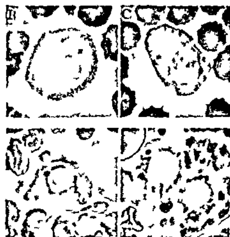


FIG 0 *b c d e* *Phase contrast pict es of plasma cells* The plasmacytoid reticulum cells show a dark drop like granulation which is much *coarser but more plentiful in a ase of m eloma lying in contact with the nuclear membrane* Lymphatic plasma cells do not show these structures It is possible that the granules represent certain protein substances formed by the reticulum cells (Zeiss phase ontrast mi ro scope 1 000)

(*b*) Lymphatic plasmoblast in the blood showing nucleoli (from a cas of Rubella)

(*c*) Mature lymphatic plasma cell in the blood (from a case of Rubella)

(*d*) Plasmacytoid reticulum cells in the bone marrow

(*e*) M eloma cells

strikingly large showing compared with the condition of the marrow a definite delay in maturation

We have only found myeloblasts in cases of leukæmia

It was previously assumed that there is a continuous development of all series from the myeloblasts and premyelocytes to the mature granulocytes that appear in the blood Rohr has asserted that the staff forms are mainly produced from metamyelocytes which in turn arise from continued division of myelocytes Thorell (1944) believes that the myelocytes are mainly produced by continued new formation and maturation of myeloblasts and premyelocytes because he was able to detect a large increased formation of plasma proteins only in these cells The complete absence of myeloblasts in the spleen seems strongly to support Rohr s view that the granulocytes are normally formed by continual division of the myelocytes

Megakaryocytes We found them in only 10 out of 220 spleen punctures (excluding the leukæmias) including 1 case of hæmolytic anæmia associated with undulant fever (up to 10 cells per slide) and also in sepsis lenta inflammatory cirrhosis 2 cases of thrombocyto penia (Fig 28) and in osteosclerotic anæmias In chronic myeloid leukæmia megakaryocytes may be scanty or occasionally numerous

D MATURE GRANULOCYTES

The distribution of the mature granulocytes in normal spleen is shown in the section on the splenogram It is however necessary to call attention to a few cells of the monocytic group with more compact nuclei and coarser granules than those found in the blood (Fig 16 *k*) These probably correspond with the reticular mono cytes (Rohr) *i e* cells which have arisen from the reticulo endothe lium perhaps through the pulp cells In cases of lymphatic reaction lymphatic monocytes are fairly frequently found These have a compact and bluish broad cytoplasm usually with few or no granules and with a general resemblance to glandular fever cells As these myeloid lymphatic and reticular monocytes in spleen punctures cannot always be distinguished from one another and as they are always rather scanty we have included them in the total number of monocytes only separating lymphatic forms in cases of glandular fever cells *i e* definitely pathological forms

E THROMBOCYTES

As Weil has previously pointed out platelets are more numerous in spleen puncture than in the blood We have tried to enumerate these elements but found that it was impossible because of very rapid coagulation The spleen seems to play a significant part in the regula tion of the platelets partly as in the case of blood cells in general

The nuclei of the young types is very characteristic The chromatin is in fine granules arranged in a delicate network in which 2 to 5 pale nucleoli are clearly visible there is a slight resemblance to the nuclear structure of young megaloblasts The nucleoli are striking because of their size and distinctness usually there are 3 or 4 large ones but occasionally there are up to 7 smaller ones With May Grunwald staining the nucleoli are pale but sharply defined (Fig 11 *a*)

Mature Lymphatic Plasma Cells Both in gland and spleen punctures it is possible to find every stage of transition between plasmoblasts and mature lymphatic plasma cells The latter differ from the plasmacytoid reticulum cells especially by their larger nucleus and finer chromatin structure (Fig 21) As a rule the younger types *i e* with remnants of nucleoli are more numerous (Fig 21 *a b c*)

C IMMATURE MYELOID CELLS FROM HÆMOPOIETIC FOCI IN THE SPLEEN

There is no necessity to describe the erythroblasts myelocytes and occasional megakaryocytes that are found in punctures of spleens in which hæmopoietic foci are present

The erythroblasts in inflamed spleens varied between 0 2–15 15 per cent and consisted mainly of polychromatic and ortho



FIG 22 Foci of ectopic myeloid hæmopoiesis

(*a*) Group of erythroblasts from the spleen in chronic inflammation (*b* and *c*) Partially mature myelocytes the former from a case of lymphatic leukæmia the latter from a hæmolytic anæmia

chromatic types (Fig 22 *a*) although many basophile ones could be found With care it is possible to find every transitional stage between the typical basophilic erythroblasts and the mature orthochromatic elements Occasionally even basophile macroblasts are seen (Fig 27 *a*)

Myelocytes (Fig 22 *b c*) are on the whole less numerous than erythroblasts in the normal spleen (0 1–0 2 per cent) In cases of inflammation they may rise to 2 7 per cent and in osteosclerosis as high as 25 per cent in the former condition the myelocytes are

G PATHOLOGICAL CELLS IN SPLEEN PUNCTURE

Descriptions of these elements will be found in the discussions of the various diseases here we only give a list of the pathological cells that may be present in spleen puncture and from which diagnostic inferences can be drawn

(1) *Pathological inflammatory cells of the reticulo endothelium*
Glandular fever cells
Epithelioid cells
Langhans giant cells } (Tuberculosis Morbus Boeck)
Bang s epithelioid cells (undulant fever)

(2) *Neoplastic cells*
Sternberg Reed cells (Hodgkin s disease)—probably neoplastic
Lymphosarcoma cells
Sarcoma cells *e g* reticulo sarcoma
Myeloma cells
Leukæmic cells—viz abnormal types *e g* para myeloblasts etc in acute and chronic leukæmias
Carcinoma cells extremely rare in the spleen but may be found if *e g* a hypernephroma or pancreatic tumour is punctured by mistake

(3) *Pathological lipoid storage cells* Gaucher and Niemann Pick diseases

H MITOSES IN SPLEEN PUNCTURES

Since Fieschi s admirable observations on cell division in the sternal marrow hæmatologists have devoted much attention to the peculiarities of mitoses and the influence of internal and external factors on them A study of mitoses in spleen puncture is also valuable particularly in the leukæmias and we would therefore first discuss the structure of the individual forms It must be admitted however that the allocation of the individual mitoses to definite cell groups requires considerable practice especially in the case of non granular forms It is specially to be noted that the cytoplasm of lymphoblasts basophile erythroblasts and cells of the reticulo endothelium is strikingly granular during the monaster and diaster stages (Fig 23 *c d i*) and it is possible therefore to confuse them with myelocytes although there are differences in the chromosomes

Mitoses in Reticulo endothelial Cells Serosa cells These elements are scanty and it is a great rarity to detect mitoses in them We were able to find a definite prophase in only 1 case (Fig 23 *a*)

Pulp Cells and Tissue Mast Cells We have never detected unquestionable mitoses in these elements

by hormonal inhibition of the marrow (Naegeli *et al*) and partly by acting as a reservoir (Heilmeyer) Probably the spleen also removes and destroys effete platelets Certainly in some forms of thrombocytopenia both inhibition of the maturation of megakaryocytes and increased destruction of platelets occur

F CELLS OF LUNG ALVEOLI

It has been mentioned that a small amount of pulmonary tissue may be aspirated *if the puncture is performed at too high a level* or if the costo pleural groove extends abnormally low This has happened three times in our experience and as the cells found in films may arouse suspicion of neoplasia it is important to recognise their features

Structure In films pulmonary alveolar cells usually form larger or smaller groups The individual cells vary from 18 to 34 μ while the boundaries are indistinct or even absent so that some of the cells seem to have 2 or 3 nuclei The nucleus (11 to 20 μ) is round and usually eccentrically placed It contains 1 2 or rarely 3 distinct nucleoli which are well demarcated from the nuclear chromatin by a dark ring The latter is fairly dense but areas of different density can be detected so that there is some resemblance to the nucleus of the mature lymphocyte The nuclear membrane is usually visible as a dark line Occasionally a small accessory nucleus is present The cytoplasm varies from reddish violet to pink and may contain a few granules of carbon or hæmosiderin (Fig 22 *d*)

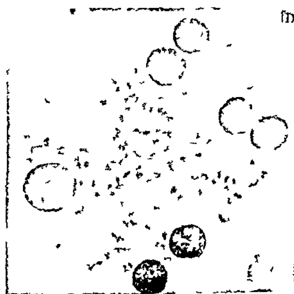


Fig 22 *d* *Cells from lung alveoli* The arrangement in smaller or larger groups and the indistinct boundaries are characteristic The nucleus is round and with small sharply demarcated nucleolus The cytoplasm is blue and in the present example contains hæmosiderin (heart failure cells)

chromosomes of lymphatic plasmoblasts (Fig 23 *b*) Plasmacytoid reticulum cells and lymphatic plasma cells are thus distinct in the structure of their mitoses

Large Lymphatic Reticulum Cells During mitosis the chromosomes are long and narrow with an acute angle (Fig 23 *e f*) They can only be distinguished from the mitoses of lymphoblasts by the size of the cells and the narrower cytoplasm No quantitative estimation of their frequency can be given because of the scantiness of these elements

Lymphoblasts The mitoses show rather delicate but short chromosomes (Fig 23 *g h i*) A typical lymphoblastic prophase is seen in Fig 23 *g* (a case of chronic lymphatic leukæmia) In comparison with the mitoses of other cell types the cytoplasm is narrow during the monaster and diaster stages

Plasmoblasts These cells with their azure blue cytoplasm and delicate dark violet elongated pairs of chromosomes in a distinct cytoplasmic rim show beautiful mitotic figures (Fig 23 *b*) and are only excelled by the delicate mitoses of megaloblasts As compared with the mitoses of myeloblasts and myelocytes the chromosomes show a fairly acute angle (Fig 23 *b*) Mitoses in such plasmoblasts are increased in infective hepatitis (6 mitoses per 100 plasmoblasts) and in chronic inflammatory spleens in sepsis lenta In gland punctures in German measles we found 1 to 3 plasmoblast mitoses per 100 lymphatic plasma cell and 1 to 6 per 100 plasmoblasts

Erythroblasts As erythroblasts are scanty in all spleen punctures *except those from cases of leukæmia only few mitoses can be found* In chronic myeloid leukæmia and particularly in so-called erythroleukæmia such mitoses are common Fieschi found 12–20 *pro mille* of erythroblast mitoses in the marrow while Rohr found figures as high as 42 *pro mille* and enumeration of the mitoses in a spleen puncture from a case of thrombocytopenia with 7 3 per cent of *erythroblasts showed the following distribution* —

	No of cells	Mitoses
Basophile erythroblasts	29	0
Polychromatic erythroblasts	30	2
Oxyphile erythroblasts	41	1
Total	100	3

The mitotic index of such extramedullary erythroblasts scarcely differs from that of the same cells in the marrow

Fieschi and Kienle have given detailed descriptions of the structure of *erythroblast mitoses* The chromosomes are broader and shorter

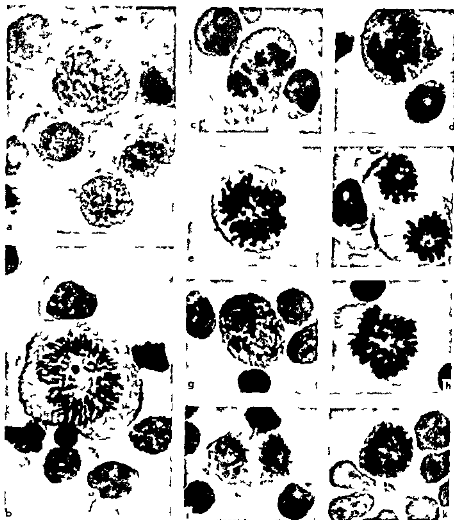


Fig 23 Mitoses in reticulo-endothelial and lymphatic cells in spleen films
(*a*) Prophase in a serosa cell
(*b*) Monaster in a lymphatic plasmoblast
(*c* and *d*) Mitoses in plasmacytoid reticulum cell
(*e* and *f*) Mitoses in large lymphatic reticulum cells
(*g* *h*) Mitoses in lymphoblasts
(*k*) Monaster in young lymphocyte (mitoses in such lymphocytes are extremely rare)

Plasmacytoid Reticulum Cells Rohr has seen unquestionable *mitoses of these cells in sternal puncture and so has controverted* the view of Klima that these elements divide by amitosis We have also seen occasional mitotic divisions in plasmacytoid reticulum cells in the spleen (Fig 23 *c d*)

The chromosomes are relatively short and wide occasional ones appearing adherent to one another These mitoses with their small chromosomes are quite distinct from the large but relatively delicate

mitoses of lymphoblasts are scarce in gland punctures but a few may be found by careful search but they are exceptional in pro lymphocytes and are almost unknown in mature lymphocytes Indeed we have only seen one metaphase (Fig 23 k) and in another case one dispireme in a young small lymphocyte This is the more remarkable because as Rohr has shown in the granulocytic series typical mitoses occur in myeloblasts and even in staff forms but most plentifully in the more mature of the myelocytes

We used films from a spleen surgically removed from a case of Felty s syndrome in which there was lymphocytic hyperplasia for comparison with films from 3 cases of lymphatic leukæmia With the assistance of Miss Ernst we obtained the following figures which are of course not to be regarded as absolute values Even in spite of the margin of error the figures emphasise the great difference in the incidence of mitoses in the lymphatic and myeloid series

Total cells examined	50 000
Lymphoblasts	12
Mitoses	2 (1 prophase 1 diaster)
Mitotic index	0 04 /

(= total of mitoses per 1 000 cells or 100 mitoses per 2½ million lymphocytes)

In the marrow Rohr found 100 mitoses in 40 000 myeloid cells and 100 mitoses in 2 400 erythroblasts *The number of mitoses in relation to the total number of lymphocytes is thus about 50 times less than in the cells of the myeloid series and 1 000 times less than in the erythropoietic series !* Even so in health about one sixth and in lymphatic leukæmia about one fifth of the lymphoblasts appear to be dividing at any one time but these elements only form 0 2–1 per cent of the lymphocytes in the normal adenogram and about 0 6 per cent in lymphatic leukæmia

Explanation presents difficulties because there is no reason to assume that the number of lymphocytes lost and destroyed is less than that of granulocytes Certainly the presence of so many lymphocytes in chronic inflammatory foci and exudates as well as in the gastro-intestinal mucosa suggests that the daily requirements of lymphocytes is high

Fieschi has suggested that amitotic division of lymphocytes may occur but we have never seen anything to suggest it In our opinion we do not believe that any proof exists for an amitotic division even in other hæmatopoietic cells

We have a rough knowledge of the life span of mature granu locytes (2–3 days according to Osgood) and of mature erythrocytes

than those of myeloid cells During prophase the cytoplasm is narrow (Fig 24 *a*) or even invisible but during the monaster and diaster stages it is obvious because of the shortness of the chromosomes (Fig 24 *b*) although less bulky than in myelocytes It is not as easy to distinguish the individual chromosomes as it is in plasmoblasts megaloblasts and even in myelocytes because they are rather stuck together Monasters in polychromatic erythroblasts are the most numerous of the mitoses

Myeloblasts and Myelocytes An occasional mitosis is seen in the myelocytes in extra medullary foci and is easily recognisable because granules are present at all stages The chromosomes are larger than those of myeloblasts and erythroblasts while they are thicker than those of



FIG 24 Erythroblast mitoses in spleen puncture from a case of erythro leukæmia
(*a*) Prophase (*b*) Monaster (*c*) Diaster (*d*) Dispireme

lymphoblasts and plasmoblasts The width of the cytoplasm is considerable in all phases of mitoses

Myeloblast mitoses which are only found in the spleen in leukæmias differ from those of erythroblasts in the more delicate and more distinct chromosomes although these are less delicate than those of lymphoblasts The cytoplasm is even narrower than in lymphoblasts during division

Spleen films are specially suitable for a study of mitoses and it may be that a comparison with those in the marrow may throw light on the development and progress of the leukæmias The effects of such therapeutic substances as arsenic urethane and X rays on mitoses can be studied by serial spleen punctures (see Leukæmia)

I THE LYMPHOCYTES THEIR MITOSES METABOLISM LIFE SPAN AND FUNCTIONS

Mitoses in the lymphocytic series are much less frequent than in the granulocytic and erythropoietic series Even in lymphatic leukæmia in which there is so great an increase of lymphocytes the scarcity of mitoses is remarkable Nordenson Klima and Fieschi mention the rarity of mitoses in the marrow in lymphatic leukæmia although they do not give any figures Fieschi goes so far as to suggest that amitotic division must occur We have noticed that

considerable life span for the lymphocytes It is conceivable that lymphocytes may return to the lymphatic tissue to regenerate their cytoplasm after having parted with various protein substances Dougherty (1946) has reached a similar conclusion Dougherty and White have suggested that lymphocytes may play a part in the production of γ globulin This is in contradiction to the work of Bing Gormsen and Fagraeus which we have already discussed (pp 14–15) which seems to have shown conclusively that this is a function of the plasma cells and not of the lymphocytes especially in so far as anti bodies are concerned

Lymphocytes probably play a significant part in digestive pro cesses as their number in the intestinal mucosa and the chyle suggests

The adrenal cortex seems to regulate both the formation and destruction of lymphocytes (Dougherty and White) a function which is of considerable significance in infections Although production of anti bodies from the lymphocytes is in Gormsen s opinion unlikely there still remains the possibility that the lymphocytes are able to produce some other globulins

K AMITOTIC CELL DIVISION

It is remarkable how uncritically and frequently amitosis has been invoked to explain the presence of two or more nuclei in a cell (Fig 17 *b* and *c*) It is true to say that we have no proof that amitosis is ever the explanation of such appearances

Confusion has doubtless been caused by the fact that even in the final phases of mitosis as seen in films of the marrow spleen and glands just before the chromosomes reunite into nuclei there may still be no signs of division of the cytoplasm This is particularly common in pathological conditions in which abnormalities of mitosis occur *e g* haploid misplaced and pyknotic chromosomes or multipolar divisions (Fieschi Rohr) in pernicious anæmia In such circumstances the nuclei may still show signs of recent division and their absence cannot therefore be regarded as evidence of amitosis All these cells with two or more nuclei are distinctly larger than the normal cells and this proves that division of the cytoplasm has not taken place

The only proof of the possibility of amitosis in hæmopoietic cells would be direct observation or a cinematographic record of its occurrence in tissue cultures Certainly Kienle s contention that the absence of chromosome structure in multi nucleated cells is strong evidence of their amitotic origin cannot be accepted especi ally as he admits that such cells are usually found in conditions in which atypical mitoses are also found (see Kienle s Figs 29 and 30) Undritz shares our view that twin and multiple nuclei are not attributable to amitotic division (polyploid cells)

(4-6 weeks according to Heilmeyer 100-120 days according to Moeschlin (8) and Rohr and others) but the life span of the lymphocytes is purely conjectural *But the infrequency of lymphocytic mitoses suggests a long life for these cells* Of course mitoses in the relatively simple lymphocytes may proceed more rapidly than in the complex granulocytes Moellendorff by a cinematographic method found that in tissue cultures fibroblasts divided in 50–70 minutes Unfortunately there are no similar investigations on lymphocytes but perhaps this problem can be solved by observations on cultures of lymphocytes

The mitoses in the spleen in lymphatic leukemia are as follows —

	A erag V I	Ca I	Ca e 2	Case 3 a	Case 3 b
				4 062	
Total lymphocytes counted	3 354	2 971	3 0_9	4 127	3 998
Of these *lymphoblasts*		20	20	20	20
In mitosis		4	0	3	2
Prophase		1	—	1	—
Monaster		3	—	2	—
Diaster		—	—	—	
Dispireme		—	—	—	—
Lymphoblasts per 1 000	6 08	6 73	6 60	4 92	
Lymphoblast mitoses per 1 000	0 65	1 34	0	0 61	
Lymphocytes per 100 lymphoblasts	16 770	14 855	15 145	?0 312	
Lymphocytes per 100 lymphoblast mitoses	121 504 (Cases I and III)	74 275	?	168 733	

The comparison of these figures with normal values shows that the mitoses are 15–30 times more frequent in Cases 1 and 3 in which lymphoblasts were more numerous Even so the relative number of lymphoblasts in mitosis viz about one fifth of the cells is approximately normal In Case 2 although the number of lymphoblasts was the same no mitoses were seen and it appears that such cases run a more prolonged course

Function of the Lymphocytes This is still obscure Sjovall (1936) investigated rabbits which had been frequently bled and reached the conclusion that there is a perpetual interchange of lymphocytes between the lymphatic tissues and the blood *i e* that the lymphocytes can return to the lymphatic tissue He did not find any increase of secondary lymph nodes and for this reason doubts whether the germ centres play any part in the production of lymphocytes It appears to us that such repeated bleedings may have induced a deficiency of some substances required for the new formation of lymphocytes analogous to the deficiency of iron for the erythropoiesis that develops in these circumstances His conclusions will therefore not stand criticism but they do indicate a

tissue penetrated is such as to ensure that most of the cells are derived from the sinuses Both sections and films of aspirated material show clearly that both pulp and follicle cells are always present Even if a small purely lymphatic follicle is spread in a film (Fig 25) little error will result if the splenogram is made by counting at least 1 000 cells in several films

The technique of spreading the material as well as that of enumeration can affect the splenogram For instance some crushed cells are found in every film especially when abnormally delicate elements are present as in the leukæmias If therefore films are not made with as little pressure as possible the proportion of the older and less fragile lymphocytes will appear unduly high The best parts of the films should be used for examination and some from the upper and lower parts as well as from the middle zone should be examined perhaps about 300 cells from each

Tempka includes crushed cells in the splenogram but in our opinion it is better not to do so if the technique advocated above is used

Table 1 shows splenograms from various cases in which counts were performed in different films from the same puncture —

TABLE 1

	Portal thrombosis		Sepsis lenta		Glandular fever		Chronic lymph leukæmia		Chronic myeloid leukæmia	
(Pp film)	Pp (*a*)	Pp (*b*)	Pp (*b*)	Pp (*a*)	Pp (*a*)	Pp (*l*)	Pp (*a*)	Pp (*d*)	Pp (*b*)	Pp (*a*)
Macrophages	—	—	0 3	0 7	+	—	—	—	—	—
Plasmacyt retic cells	0 3	0 1	3 0	1 0	1 1	1 4	—	—	—	—
Pulp cells	0 5	0 9	1 5	0 8	1 0	0 5	—	—	—	—
Erythroblasts basophiles	0 1	—	—	—	—	—	—	—	0 6	0 7
Erythroblasts polychr	—	—	±	—	0 1	—	—	—	3 1	3 5
Erythroblasts orthochr	—	—	—	—	—	—	—	0 1	4 9	1 9
Myeloblasts	—	—	—	—	—	—	—	—	2 4	0 6
Myelocytes immature	—	—	—	0 1	—	—	—	—	11 0	13 8
Myelocytes half mature	—	—	0 2	0 [illegible]	—	0 1	0 1	—	2 0	22 7
Myelocytes mature	0 2	—	0 6	0 2	—	—	0 1	0 1	3 6	1 4
Metamyelocytes	—	—	0 6	0 4	0 1	0 1	—	—	14 6	11 7
Neutrophiles staff	4 0	5 6	7 9	9 6	7 4	8 8	0 3	0 3	2[illegible] 4	26 0
Neutrophiles segmented	12 9	14 6	6 2	7 9	4 0	4 4	1 2	2 1	10 4	6 8
Eosinophiles mature	2 3	1 4	—	0 1	0 2	0 7	—	—	1 1	1 7
Basophiles mature	0 2	0 3	0 1	0 2	0 2	0 2	0 1	0 1	4 0	2 6
Monocytes	2 7	2 8	0 7	1 9	4 6	4 2	0 2	0	0 5	0 8
Lymphoblasts	0 1	—	1 3	0 8	—	—	0 8	0 5	—	—
Lymphocytes small y ng	10 5	5 8	5 7	8 6	2 5	1 9	29 [illegible]	3[illegible] 4	—	—
Lymphocytes small old	64 1	63 3	57 6	45 5	4[illegible] 4	46 6	65 2	59 3	1 4	0 7
Lymphocytes large y ng	0 8	0 9	6 2	8 3	0 1	0 6	1 4	3 2	—	—
Lymphocytes large old	1 1	4 1	5 7	10 0	5 9	6 8	1	1 7	—	0 1
Lymphocytes total	76 6	74 1	76 5	73 2	78 6	78 1	98 0	97 1	1 4	0 8
Lymphoid monoblasts	—	—	—	—	4 5	1 1	—	—	—	—
Glandular fever mature cells	—	—	—	—	23 2	21 1	—	—	—	—
Plasmoblasts	+	—	0 3	0 5	0 1	—	—	—	—	—
Plasma cells half mature and mature	0 2	0 2	2 1	3 7	2 6	1 5	—	—	—	—

I. THE SPLENOGRAM

When spleen puncture is performed purely for diagnosis it usually suffices to examine the films carefully without making a detailed differential count In such conditions as aleukæmic lymphatic leukæmia with splenomegaly (splenomegalic lymphadenosis) it is advisable to obtain a full qualitative picture i e a splenogram Then again in myeloid leukæmia a splenogram may be useful for comparison with the myelogram

A considerable number of splenograms are recorded in Part II mainly for didactic purposes

The earliest splenograms were published by Mele later ones by

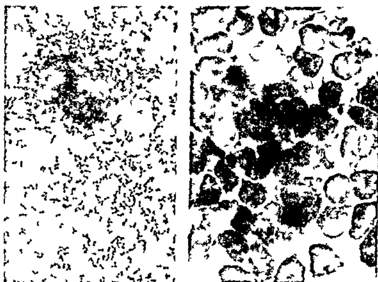


Fig 25 Lymphatic follicle in spleen film low and high magnification

Weil and normal figures by Tempka We have evaluated many splenograms and are convinced that at least 1 000 cells must be examined and classified while in order to diminish the margin of error as much as possible certain differential features must be kept in mind

If our technique of puncture is properly followed the small amount of blood in the spleen juice is derived from the pulp sinuses This does not cause confusion because the sinuses are not normally connected with the general circulation their cellular contents being typically splenic It is only when there is a marked degree of portal hypertension that pure blood from the dilated sinuses enters the syringe Even so in every case the splenogram should be judged in the light of the blood picture as a whole

There is little likelihood of obtaining only material from a lymphatic follicle the size of the needle and the depth of spleen

TABLE 4

Variations of Normal Splenogram

			Weil		*Tempka*	
Reticulum cells						
Pigment and cell macrophages	0 1–0 3	0 5–1 8	Medium mononuclears	10– 0	Reticulum cells	0 6–? 5
Fat cells (1 per 10 000)	0–0 01				Endothelial cells	0 3
Plasmacytoid reticulum cells	0 2–0 8				Monocytoid cells	0 4 1 15
Tissue mast cells	0–0 1					
Pulp cells	0 2–0 6					
Erythroblasts						
Basophilic polychromatic orthochromatic		0–0 1–0 .	Erythroblasts	0 2	Erythroblasts	0
Granulopoiesis						
Myeloblasts	0				Myeloblasts	0
Myelocytes immature	0–0 1	*0 05–0 3*				
Myelocytes half mature	0–0 1				Myelocytes	0
Myelocytes mature	0 05–0					
Metamyelocytes	0–0 1					
Neutrophiles staff	1 0–7 0	*11–31*	Neutrophiles	.0–30	Neutrophiles staff	0 45 1 65
Neutrophiles segmented	8–25				Neutrophiles segmented	5 8
Eosinophiles mature	0 2 1 5		Eosinophiles	1	Eosinophiles	0 12–0 6
Basophiles mature	0 1 1 1		Basophiles	rarely	Basophiles	0 1?–0 5
Monocytes	1 ?–2 4		Monocytes	5– 0	Monocytes	0 5
					Degenerate granulocytes	0 5 8 6
Lymphatic series						
Large lymphatic reticulum cells		0–0 1				
Lymphoblasts		0–0 2			Lymphoblasts	0 3–7 0
Lymphocytes					Lymphocytes	41–59 5
Small young	0 6–7 5	1 0–10 5				
Large young	0 4–3 0					
Small mature	55–79 5	57 84 5	Lymphocytes (typical)	50–60	Lymphocytes pseudo	1 15–11 4
Large mature	2–5	*58–89*			Lymphocytes, degenerate	18 5–4? 1
Lymphatic plasma cells						
Plasmoblasts	0–0 5	0–0 3	Plasma cells	1–2	Plasma cells	0 5 0 83
Lymphatic plasma cells (mature)	0–0 3					
Megakaryocytes		0			Unclassifiable	0 15–2 0

The splenogram shows that the percentage variations of the lymphocytes is only $\pm$ 3 3 and that of the neutrophiles $\pm$ 4 7

In the following case we performed two punctures on a con siderably enlarged spleen one at the upper pole and the other in the lower third and made separate splenograms from them (Table 2)

TABLE 2

	Splenogram																		
	Macrophages	Plasmacyt retic cells	Pulp cells	Tissue mast cells	Erythroblasts	Myelocytes	Neutrophiles staff	Neutrophiles segmented	Eosinophiles	Basophiles	Monocytes	Lymphoblasts	Lymphocytes small young	Lymphocytes small old	Lymphocytes large young	Lymphocytes large old	Lymphocytes Total	Plasma cells
I Medial	0 9	3 0	0 6	0 1	1 8	0 3	7 2	23 9	0 1	0 2	4 0	0 1	5 1	48 9	1 9	1 6	57 6	0 3
II Lateral	0 9	2 9	0 2	—	1 1	0 8	6 7	24 3	0 1	0 4	4 7	0 1	5 3	45 9	3 4	3 1	57 8	0 1

TABLE 3

Comparison of Puncture and Operation Films

Case	1			2		3		
	Punc ture	Punc ture	*Film*	Punc ture	*Film*	Punc ture	Punc ture	*Film*
Date	1/41	9/42	10 43	[illegible]/44	3/44	5/4[illegible]	1[illegible]/44	1/45
Macrophages	—	—	0 1	—	—	[illegible]	0 [illegible]	0 1
Plasmacyt retic cells	0 2	1 0	0 1	0 9	0 3	0 2	0 3	0 4
Pulp cells	0 2	*1 0*	*0 4*	*1 8*	0 5	—	0 2	0
Tissue mast cells	—	—	0 1	—	—	—	—	
Erythroblasts	—	—	—	—	0 1	—	—	—
Myelocytes	—	0 1	—	0 3	0 2	—	—	—
Neutrophiles staff	6 6	5 8	3 4	7 0	4 0	2 9	0 1	0 3
Neutrophiles segmented	21 3	5 4	5 8	[illegible] 2	1[illegible] 9	3 [illegible]	0 1	0 6
Eosinophiles	2 4	2 3	1 5	2 3	2 3	4 3	4 1	2 2
Basophiles	0 9	0 3	0 3	0 6	0 2	0 3	0 4	0 7
Monocytes	3 8	2 0	2 7	4 1	2 7	8 3	3 9	6 4
Lymphoblasts	—	0 2	0 5	0 1	0 1	0 3		0 1
Lymphocytes small young	2 0	7 6	8 8	1 6	10 5	7 6	4 8	10 9
Lymphocytes small old	60 1	71 9	64 7	55 7	64 1	69 6	81 0	71 4
Lymphocytes large young	—	1 0	5 8	0 3	0 8	1 9	1 5	1 6
Lymphocytes large old	2 5	1 2	5 5	2 7	1 1	1 2	[illegible] 9	4 6
Lymphocytes total	64 6	81 9	85 3	60 4	76 6	80 6	90 2	88 6
Plasmoblasts	—	—	0 1	—	—	—	—	0 1
Plasma cells half mature and mature	—	0 [illegible]	0 2	0 4	0 2	0 2	0 [illegible]	0 4

Case 1 splenomegalic cirrhosis Case 2 thrombosis of splenic vein Case 3 Felty s syndrome

of individual Splenograms

Chronic inflammatory	Lymphatic leukæmia	Myeloid leukæmia
10 20 30 40 50 60 70 80 90 100	10 20 30 40 50 60 70 80 90 100	10 20 30 40 50 60 70 80 90 100

the latter case the lymphocytes were 5–16 per cent higher. Table 3 shows the results of such parallel investigations of 3 cases.

All these observations support the view that the qualitative picture obtained by spleen puncture accurately represents the distribution of free cell types in the spleen.

We have already pointed out that the reticulum cells of the spleen stroma are found in only small numbers because they form a firm syncytium. For this reason far larger numbers of these cells are found in sections than in films, as is also the case in the sternal marrow (Rohr). This fact is of no importance for clinical and diagnostic purposes because the significant variations affect the free cells of the organ.

The Normal Splenogram. The variations of the normal splenograms are shown in Table 4. As only definitely enlarged spleens are punctured, and as normal spleens are only rarely removed by operation, it is not strictly true to say that the normal splenogram is known. Hellmann's histological investigations suggest that the number of lymphocytes in the spleen diminishes with increasing age, but our punctures of pathological spleens do not give this impression.

For differential diagnosis the most important feature is the

FIG 26a *Graphic representatio*

	Normal values	Normal 10 20 30 40 50 60 70 80 90 100	Acute inflammatory 10 20 30 40 50 60 70 80 90 100
Macrophages	0 1–0 3		
Plasmacyt retic cells	0 2–0 8		
Pulp cells	0 2–0 6		
Tissue mast cells	*0–0 1*		
Erythroblasts	0–0 1–0 2		
Myeloblasts	0		
Myelocytes immature	0		
Myelocytes ½ mature	(+)		
Myelocytes mature	0 05–0 1		
Metamyelocytes	0–0 1		
Neutrophiles staff	1 0–7 0		
Neutrophiles segmntd	8–25		
Eosinophiles mature	0 2–1 5		
Basophiles mature	0 1–1 1		
Monocytes	1 2–2 4		
Lymphoblasts	0–0 2		
Lymphocytes sm yng	0 6–7 5		
Lymphocytes large yng	0 4 3 0		
Lymphocytes sm old	55–79 5		
Lymphocytes large old	2–5		
Plasmoblasts	0–0 05		
Lymphatic plasma cells mature	0–2 3		
Megakaryocytes	0		

We were surprised at the slight variations of the values and it is justifiable to draw the conclusion that splenograms obtained by spleen puncture give a relatively accurate picture of the cellular composition of the spleen but, of course only if there is no con siderable admixture of blood Thus as in the case of myelograms the findings can be directly compared with one another

We have expressed all the values as percentages which are based on examination of at least 1 000 cells In the case of myelograms Rohr enumerates the white cells separately and then mentions the number of reticulum cells and erythroblasts seen while counting each 100 white cells *This method has the advantage of permitting the values found* for the granular series to be compared with the blood picture In the spleen where lymphocytes normally preponderate very greatly this method of differentiation serves no purpose and equally it is of no value to indicate the number of other types of cells per 100 lymphocytes

On a number of occasions we have compared splenograms made from puncture during life and also from films made directly after splenectomy and we were surprised how little they differed from one another The main difference occurred if the spleen had been com pressed during the operation or if the films were made some time *after* incising the organ (loss of blood from the pulp sinuses) In

through the splenic artery contained 350 000 platelets per c mm while that leaving in the splenic vein contained only 17 000 In 9 cases in which splenectomy was performed he found a distinctly raised lecithin level this may have played a part in the increased destruction of blood cells

Many observations seem to support the view that the spleen exerts an hormonal effect on the marrow even although no proof is yet available Even so the rapid rise of reticulocytes and the emigration of cells from the marrow even during the first few hours after removal of the spleen seems to be best explained by such a mechanism

Then again the concept of a correlation between lymphatic and myeloid tissues has much to commend it For instance Miller and his colleagues found a substance in the urine of patients with lymphatic leukæmia which was capable of inhibiting myelopoiesis while the converse was also found to be the case It would seem reasonable to suppose (Rohr) that great lymphatic hyperplasia in the spleen (as in Felty s syndrome) might in a similar way have an inhibitory action on the marrow Perhaps the anæmia and granulocytopenia found in some chronic lymphatic leucosis is partly due to the same mechanism and not only to infiltration of the marrow by leukæmic cells because this infiltration may be absent in spite of marked anæmia and granulocytopenia Certainly the latter is not always demonstrable in anæmic and granulocytopenic cases of lymphatic leukæmia

Occurrence of Hypersplenism Hypersplenism can occur in association with any type of splenomegaly and we shall refer to this in the appropriate sections Here only a list (in order of frequency) will be given —

(1) Non inflammatory splenomegalies
- Splenomegalic cirrhosis
- Thrombosis of splenic vein
- Hæmolytic anæmias
- Storage diseases

(2) Inflammatory diseases of the spleen
- Chronic malaria
- Felty s syndrome
- Splenic tuberculosis
- Syphilis
- Kala azar
- Sarcoidosis (Boeck)

(3) Neoplastic
- Lympho sarcoma
- Sarcoma
- Primary malignant reticuloses
- Hodgkin s disease

maximum value of the lymphocytes as well as the maximum values of inflammatory cells viz staff forms polymorphs and monocytes We have given the figures of Weil and Tempka for comparison with our normal values although the difference of terminology makes this rather difficult

M HYPERSPLENISM AND INHIBITION OF THE MARROW

Naegeli stressed the intimate relation between spleen and marrow recognising the condition of hypersplenic inhibition of the marrow which he attributed to an endocrine action of the enlarged spleen This idea has been accepted by a number of other writers *e g* Rohr Heilmeyer Mallarme Doan Dameshek Hittmair etc

Clinical Features Hypersplenism is not a disease *sui generis* but is a syndrome There is always some degree of splenomegaly which is accompanied by a more or less severe normochromic or hyperchromic anæmia with well marked granulocytopenia and sometimes by thrombocytopenia Usually the whole hæmopoietic tissue is affected but one component may be more so than the others

The bone marrow is usually hyperplastic but in the later stages may become hypoplastic The myelogram shows a shift to the left *i e* a relative increase of immature myelocytes and basophile erythroblasts sometimes with macroblasts and an increase of megakaryocytes some being of young type In other words marrow puncture reveals *a picture of marrow inhibition with delayed cellular maturation i e* shift towards immaturity and a tendency to the development of large cells This explains the characters of the blood picture in such cases but occasionally the marrow does not show a leftward shift and it is clear therefore that the idea of an endocrine inhibition of the marrow by the enlarged spleen is not a complete explanation of the hypersplenic syndrome

Ætiology There are three possibilities —

(1) Increased destruction of cells in the enlarged spleen

(2) Endocrine inhibition (splenic in origin) of the marrow

(3) Excessive lymphocytopoiesis in the enlarged spleen and perhaps also in the other enlarged lymphatic tissue with consequent disturbance of the correlation between the lymphatic and the myeloid systems

It is probable that all three factors are concerned in the genesis of the syndrome The increased destruction may be due to delay of the cells in the enlarged spleen thus adding to the chance of lysis Thus it is known that the spleen can destroy blood cells in virtue of its possession of hæmolysins (see chapter on hæmolytic anæmias) and it is probable that it can remove leucocytes and platelets by a similar mechanism Doan described a typical case of the hypersplenic syndrome in which the blood entering the spleen

PART II SPECIAL

THE SPLENOGRAM IN SPLENOMEGALIES

We have discussed the various forms of splenomegaly from a clinical and diagnostic point of view rather than from a strictly pathological one

A NON INFLAMMATORY HYPERPLASIAS OF THE SPLEEN

In this group fall those diseases in which enlargement of the spleen is due to increased functional demand and also to stasis in the portal circulation *e g* splenomegaly in hepatic cirrhosis thrombosis of the splenic or portal veins and the hæmolytic anæmias

Splenomegaly due to compensatory revival of hæmopoiesis in the spleen also falls into this group

I CIRRHOSIS OF THE LIVER

Spleen puncture is only of diagnostic value in those cases in which enlargement of the spleen dominates the clinical picture sometimes for years before hepatic disease is detectable (Heilmeyer)

In such cases differential diagnosis may lie between chronic inflammatory splenomegaly aleukæmic lymphadenosis isolated Hodgkin s disease of the spleen and a storage disorder *e g* Gaucher Here spleen puncture will be valuable because the splenogram associated with hepatic cirrhosis unlike that in most other diseases is practically normal

Some degree of splenic enlargement is discoverable in all cases of hepatic cirrhosis although the cause of this is still not known with certainty Sternberg incriminates the same agent that causes the cirrhosis attributing much less importance to stasis in the portal circulation Herxheimer admits the importance of stasis but asserts that there is also genuine hyperplasia of the spleen Probably these are not the only relevant factors For instance the splenomegaly that occurs in Kinnier Wilson s disease suggests the importance of nervous influences while some unknown noxa has to be postulated in those cases in which great enlargement of the spleen precedes the development of hepatic cirrhosis sometimes for years

We have seen the syndrome in association with splenomegalic cirrhosis (p 55) in one case of hæmolytic familial jaundice lymphosarcoma and one case of reticulosis (p 184) Wintrobe has recorded an instructive case of the syndrome in association with syphilis of the spleen (see also Curschmann) Cartwright has discussed its occurrence in kala azar Many other references will be found in the relevant literature especially Doan Mallarme and Dameshek

Some degree of marrow inhibition presumably of splenic origin also occurs in chronic myeloid leukæmia (see our discussion of this fact in the chapter on leukæmias)

Treatment The condition may be so severe as to make treatment imperative especially in the dangerous cases with severe thrombocytopenia The following is a list of the more important indications —

(1) Cure of the infective cause of the splenomegaly *e g*, malaria syphilis kala azar tuberculosis

(2) X irradiation when removal is not possible or the condition is neoplastic *e g*, Hodgkin s disease sarcoma primary reticulosis

(3) Splenectomy

Specific treatment may cure the syndrome *e g* in malaria kala azar and syphilis In primary reticulosis with extreme leucopenia (500–720 per c mm) and an hæmorrhagic diathesis we saw great improvement after careful radiotherapy to the spleen (p 185) A similar effect may be obtained in severe thrombocytopenia when splenectomy is contra indicated *on account of an acute exacerbation In all other cases splenectomy is the treatment of election*

It is particularly in case of hypersplenic inhibition of the marrow that spleen puncture is of the greatest value in diagnosis and in indicating the most suitable line of treatment

for the production of some globulins especially when the increased number of plasmacytoid reticulum cells is combined with a remark able enlargement of this organ as in splenomegalic cirrhosis and in chronic inflammatory conditions

In the second case there is a combination of alcoholic cirrhosis with a secondary hæmolytic anæmia (according to Eppinger by no means a rarity) The spleen was enlarged (23 cm) and puncture again revealed an increase of plasmacytoid reticulum cells probably as in the first case attributable to the hepatic changes The large number of pigment macrophages (1 8 per cent instead of 0 1 per cent) was presumably a consequence of the increased hæmolysis while the erythroblastosis (5 8 instead of 0 1 per cent) and the augmentation of the number of myelocytes (1 6 per cent instead of 0 1-0 2 per cent) is usual in hæmolytic diseases (p 69)

2 SPLENOMEGALIC CIRRHOSIS

For clinical purposes it is convenient to distinguish these cases from the typical examples of cirrhosis Unlike Laennec s cirrhosis which is usually due to alcohol (Wuhrmann and Constam) the ætiology of splenomegalic cirrhosis is unknown but certainly usually occurs at an earlier age (Heilmeyer) Such authorities as Heilmeyer Weil Fanconi etc believe that a condition worthy of being called Banti s disease does exist although most writers would grant it only the status of a syndrome (Jentzer and Weyeneth)

The term is applicable to primarily chronic splenomegaly possibly of varying ætiology which is often followed by hepatic cirrhosis and in which anæmia leucopenia and thrombocytopenia occur early *i e* an anæmia of Naegeli s type of hypersplenic inhibi tion of the marrow Bleeding from the gastro intestinal tract may precede the development of cirrhosis but splenectomy in the early stages can produce permanent cure (Heilmeyer)

The following is a typical example —

B F aged 38 male

History Vague abdominal discomfort at age 23 appendectomy—operation (Billroth II) a year later but discomfort persisted In 1940 (age 34) diarrhœa meteorism and anæmia (hæmoglobin 59 per cent) with moderate ascites No history of alcohol or of jaundice Liver not enlarged Spleen (7 cm) firm and easily palpable Hæmoglobin 72 per cent red corpuscles 3 600 000 per c mm leucocytes 3 000 per c mm platelets 40 400 per c mm Thus a typical splenopathic inhibition of the marrow During the next four years the spleen became progressively larger (23 cm) and there was occasional melæna The hæmoglobin fell to 59 p r cent there was no improvement with liver extracts and only slight effect from iron

After 3 years (1943) it was decided to perform splenectomy and a Talma operation There was typical nodular cirrhosis (biopsy) Spleen 750 g 23 cm. No ascites The operation was followed by prolonged pyrexia and ascites and pleural effusion gradually developed Death

1 LAENNEC S CIRRHOSIS

Table 5 shows the splenograms of 2 cases The first case was a typical alcoholic cirrhosis (Takata+++ S R 35 bilirubin 1 8 prothrombin 30 per cent) and a definitely palpable spleen (17 cm) The sternal marrow showed a marked increase of plasmacytoid reticulum cells (34 per 100 leucocytes) and the spleen showed a similar increase (4 3 per cent) The normal for inflammatory conditions is only 0 2–0 8 per cent and the highest values in a chronic inflammatory splenomegaly (Felty s syndrome) were 10 3 per cent and 17 per cent The splenogram also shows an increase of neutrophiles and monocytes both of which are probably due to portal stasis and therefore congestion of the red pulp Pigment macrophages but no other phagocytes were scanty while the pulp cells were somewhat increased

TABLE 5

Case number	1	2	Case number	1	2
Age Sex	54♂	50♂	Erythroblasts	—	5 8
Hæmogram			Myelocytes	0 2	1 6
Leucocytes (in 1 000s)	6 2	3 2	Neutrophiles staff	4 2	5 0
Neutrophiles staffs	17	22	Neutrophiles segmented	23 7	3 9
Neutrophiles segmented	50	25½	Eosinophiles	2 3	1 4
Eosinophiles	½	4	Basophiles	0 2	0 5
Basophiles	2	1	Monocytes	7 3	1 8
Monocytes	17½	6½	Lymphoblasts	—	0 1
Lymphocytes	13	41	Lymphocytes small young	4 4	4 3
			Lymphocytes small old	47 5	68 5
Splenogram			*Lymphocytes large young*	*1 0*	*0 7*
Macrophages	0 1	1 8	Lymphocytes large old	2 3	0 6
Plasmacyt retic cells	4 3	2 4	Lymphocytes Total	55 2	74 1
Pulp cells	2 5	0 1	Plasma cells	—	0 8

Rohr Klima Fleischhacker Wuhrmann and others have pointed out the relationship between the raised plasma globulin (according to Wuhrmann and Wunderly who used the electrophoretic method mainly β and γ globulin) and the increase of plasmacytoid reticulum cells The neoplastic cells of plasmacytomas usually produce γ globulin but only in exceptional cases the β type Wuhrmann inclines to the view that the dysproteinæmia is primary and the increase of plasmacytoid reticulum cells secondary (further details see p 14) We adhere to the idea of the formation of certain globulin fractions by these cells perhaps however only in the sense of Brass s protein transformation

In the above case of advanced cirrhosis with strongly positive Takata reaction and greatly increased sedimentation puncture revealed as great an increase of plasmacytoid reticulum cells in the spleen as in the marrow

If then these elements are admitted to be producers of certain globulins it is to be expected that even the spleen is of importance

TABLE 6b

Case	Macrophages	Plasmacytoid retic cells	Pulp cells	Erythroblasts: Basophiles	Erythroblasts: Polychrom	Erythroblasts: Orthochro	Myelocytes	Neutrophiles: Staff forms	Neutrophiles: Polymorphs	Eosinophiles	Basophiles	Monocytes	Lymphocytes	Plasma cells	Megakaryocytes: Half mature	Megakaryocytes: Mature
1 47 ♀				1 2	2 3	0 [illegible]		2 1	7 [illegible]							
	0 3	0 4	0 8	3 7			1 2	9 3		0 7	0 5	2 0	79 9	1 2	—	—
2 37 ♀				5 1	10 0	0 2		10 8	28 1							
	0 6	0 9	2 6	15 3			0 5	38 9		5 7	2 0	4 2	29 8	—	+ +	– +
3 39 ♂								13 0	21 7							
	0 3	1 3	0 3	0 3			2 0	34 7		4 0	0 3	4 0	57 6	—	+ +	(+

In Case 2 a 37 year old woman presented the picture of severe cirrhosis with ascites (plasma bilirubin 2 0 mg per cent Weltmann 0 15 widened prothrombin 50 mg per cent Takata and Cephalin + +) The diagnosis was histologically confirmed In spleen films up to 15 per cent of basophile macroblasts were found together with distinct neutrophile reaction

In the third case a man of 39 the clinical features were those of early cirrhosis (Weltmann widened 0 1 Takata and cadmium + prothrombin 60 per cent fibrinogen 0 5 mg per cent) although histological examination of the liver was not possible The spleen contained a few erythroblasts and numerous megakaryocytes

These 3 cases might probably show that the presence of erythroblasts and megakaryocytes in spleen puncture are consistent with the presence of inflammatory cirrhosis of the liver (alcoholic origin could be excluded in all 3 cases) if the spleen changes are not due to the occurrence of hæmorrhage Of course more cases are needed for confirmation of these inferences especially for the determination of whether the same agent that causes the cirrhosis also causes the development of the ectopic foci of hæmopoiesis The presence of megakaryocytes is of special interest

(*b*) *Schistosomiasis* For the sake of completeness we will mention that in Egyptian splenomegaly the picture may resemble splenomegalic cirrhosis in which thrombosis of the splenic vein may occur in the late stages Wintrobe asserts that the only distinctive feature is the presence of the parasites in the stools The disease produced by schistosomiasis mansoni or japonicum is not only found in the East but also in South and Central America

occurred 1½ years later At autopsy healed (post operative) peritonitis *small nodular cirrhotic liver but no thrombosis of portal or splenic veins*

TABLE 6a

Date	1/41	11/42	10/43	Date	1/41	11/42	10/43
	Puncture		Film *		Puncture		Film *
Hæmogram				Tissue mast cells	—	—	0 1
Leucocytes (in 1 000s)	3 1	2 9	2 4	Myelocytes	—	0 1	—
Neutrophiles staff	25½	24½	35	Neutrophiles staff	6 6	5 8	3 4
Neutrophiles				Neutrophiles segmented	21 3	5 4	5 8
segmented	25	32½	38	Eosinophiles	2 4	2 3	1 5
Eosinophiles	8½	11	6½	Basophiles	0 9	0 3	0 3
Basophiles	2½	1	½	Monocytes	3 8	2 0	2 7
Monocytes	*5½*	*10½*	*5½*	*Lymphoblasts*	—	*0 2*	*0 5*
Lymphocytes	33	20½	14½	Lymphocytes small young	2 0	7 6	8 8
				Lymphocytes small old	60 1	71 9	64 7
Splenogram				Lymphocytes large young	—	1 0	5 8
Macrophages	—	—	0 1	Lymphocytes large old	2 5	1 2	5 5
Plasmacyt retic cells	0 2	1 0	0 1	Lymphocytes Total	64 6	81 9	85 3
Pulp cells	0 2	1 0	0 4	Plasma cells	—	0 2	0 3

After splenectomy

This is clearly a case of splenomegalic cirrhosis which at first simulated a case of thrombosis of the splenic vein The splenogram was normal and inflammatory neoplastic and leucotic conditions could therefore be excluded Even a later splenogram (1942) was normal indeed the resemblance of the two from *in vivo* punctures *and one made from films at operation is very striking (Table 6a)* Perhaps the rather raised lymphocyte value can be regarded as more suggestive of splenomegalic cirrhosis than of portal thrombosis possibly being part of the general effect of splenic activity on hæmopoiesis

Even when the splenogram is normal it is very important to *differentiate between splenomegalic cirrhosis and portal thrombosis* because the earlier splenectomy is carried out in the former the better the outlook *e g* Heilmeyer has recorded cure in a boy of 15 whereas the expectation of life is otherwise about 7 years Torrioli and Pusic recorded 4 cases with 2 cures 1 of more than 5 years duration Perhaps some inflammatory cases may be outlined from *this idiopathic form*

(*a*) *Splenomegalic Cirrhosis of Inflammatory Origin* In 2 cases liver biopsy revealed the typical appearances of inflammatory cirrhosis with much interstitial inflammation increase of connective tissue proliferation of lymphocytes and plasma cells

The splenogram is given in Table 6b

Case 1 was a woman aged 47 who complained of vague abdominal discomfort and whose spleen was distinctly enlarged while the plasma bilirubin was raised to 1 8 mg per cent Spleen puncture revealed 3 7 per cent erythroblasts and occasional myelocytes but no megakaryocytes

numerous varicosities in the lower third Serum iron 22 gamma per cent Prothrombin concentration reduced = 45 improvement after administration of iron and blood transfusion Operation was refused and the patient remained well until January 1944 when there was again severe bleeding The spleen was now 20 × 14 cm smooth and hard with little movement on respiration Liver not enlarged No collateral circulation observable on the abdominal wall Hæmoglobin 33 per cent red corpuscles 2 300 000 reticulocytes 10 3 per cent leucocytes 2 600 with 8 per cent lymphocytes platelets 18 000 cholesterol reduced 56 serum iron 30 Takata negative bilirubin 0 6 protein not reduced = 6 7 prothrombin 40 mg per cent

Œsophageal varices were very well marked but no radiographic changes in the stomach

Sternal puncture showed increased erythropoiesis with slight general immaturity (Spleen puncture see Table 8) Blood transfusion and

TABLE 8

Date	2/44	3/44	Date	2/44	3/44
	Puncture	Film		Puncture	Film
Hæmogram			Myelocytes	0 3	0 2
Leucocytes (in 1 000s)	2 8	3 3	Neutrophiles staff	7 0	4 0
Neutrophiles, staff	16	11	Neutrophiles segmented	72 2	12 9
Neutrophiles segmented	43	56	Eosinophiles	7 3	2 3
Eosinophiles	3	2½	Basophiles	0 6	0·2
Basophiles	3½	½	Monocytes	4 1	2 7
Monocytes	12½	7½	Lymphoblasts	0 1	0 1
Lymphocytes	22	22½	Lymphocytes small young	1 6	10 5
			Lymphocytes small old	55 7	64 1
Splenogram			Lymphocytes large young	0 3	0·8
Plasmacyt retic cells	0 9	0 3	Lymphocytes large old	2 7	1 1
Pulp cells	1 8	0·5	Lymphocytes Total	60 4	76 6
Erythroblasts	—	0 1	Plasma cells	0 4	0

administration of iron raised the hæmoglobin to 85 per cent A diagnosis of thrombosis of the splenic vein was made

Operation was performed in the Surgical Clinic of Professor Brunner On March 7th 1944 the spleen 21 × 12 cm weighed 750 g There was a mass of veins as thick as the thumb at the upper pole of the spleen and collateral veins as thick as a pencil running to the diaphragm Removal of the spleen was difficult because of numerous adhesions and the exact condition of the splenic vein could therefore not be determined but there was no thrombosis in the hilum liver and portal veins appeared normal

Histological examination of the spleen in the Pathological Institute in Zurich (Professor v Meyenburg) showed dilated sinuses similar to the condition seen in portal stasis but no other abnormalities were detected

In the autumn of 1944 the general condition was good hæmoglobin 90 per cent leucocytes 8 400 platelets 120 000 occasional Howell Jolly bodies in the red corpuscles such as are usually found after splenectomy The œsophageal varices were distinctly smaller but were still detectable

This case shows a typical picture of thrombosis of the splenic

3 WILSON S DISEASE

Very large spleens may be found in this condition (Klemperer) but at this stage cirrhosis and other clinical features are fully developed and spleen puncture is of no diagnostic importance In a typical case in a 30 year old man with ascites and a large spleen puncture showed the following splenogram (Table 7)

TABLE 7

Hæmogram								Splenogram											
Total leucocytes (in 1 000s)	Neutrophiles staff	Neutrophiles segmented	Eosinophiles	Basophiles	Monocytes	Lymphocytes	Plasma cells	Plasmacytoid retic cells	Pulp cells	Fat cells	Neutrophiles staffs	Neutrophile segmented	Eosinophiles	Basophiles	Monocytes	Lymphocytes small	Lymphocytes large	Lymphocytes Total	Plasma cells
2 8	13	46	6	1½	10	23	½	0 7	2 5	0 1	6 9	22 0	8 1	0 9	3 6	54 3	0 5	54 8	0 4

There was well marked eosinophilia (8 1 per cent as compared with the normal 0 2–1 5 per cent) which was also noticeable in the blood (6 per cent) Death occurred a year later in spite of a Talma operation Autopsy confirmed the diagnosis but did not reveal any noteworthy histological changes in the spleen (740 g)

4 HÆMOCHROMATOSES

In one case pigment macrophages were very numerous but as there was a good deal of blood mixed with the spleen juice the percentage could not be evaluated In one case of secondary hæmo chromatosis with splenomegaly which occurred after many blood transfusions in a case of aplastic anæmia hæmosiderin macrophages were numerous (0 6 per cent) (Moeschlin and Rohr 8)

II THROMBOSIS OF THE SPLENIC AND PORTAL VEINS

Eppinger has paid much attention to the thrombosis of the splenic vein the symptoms of which were clearly described by Naegeli (2)

We have details of 3 cases —

Case 1 B G aged 21 female shop assistant

At the age of 9 suspected appendicitis—no operation but at the age of 18 (1941) sudden severe gastro intestinal hæmorrhage

The spleen was enlarged (11 cm) but the liver was not Hæmoglobin 45 per cent red corpuscles 2 600 000 per c mm leucocytes 2 300 per c mm platelets 47 000 X ray examination of the œsophagus showed

reactive formation of extramedullary foci can be so great as to produce distinct enlargement of the spleen Probably the immature cells never belong to one series only granulocytes and erythropoietic elements occur together although one may predominate The following types can therefore be distinguished —

(1) Mixed myelocytic erythroblastic types
  (a) Compensatory
  (b) Chronic inflammatory
  (c) Leukæmic
(2) Predominantly erythroblastic types
(3) Cases with numerous megakaryocytes

(1) *Mixed Myelocytic Erythroblastic Types* Splenomegaly which is mainly due to the presence of extra medullary hæmopoietic activity can occur as a compensatory phenomenon in association with all those conditions in which the marrow is extremely reduced in amount but also in chronic inflammatory states and in leukæmias where it may be neoplastic

(a) *Compensatory Types* Here a typical example is found in marble bone disease so called osteosclerotic anæmia in which the bone marrow is progressively reduced in amount and ultimately almost destroyed by osteosclerosis Extra medullary foci of blood formation develop in the liver and in the spleen often with considerable increase in size of both organs (Naegeli) Similar changes may occur with extensive deposits of tumour in the bone marrow e g carcinomatosis sarcomatosis and multiple myeloma We saw a case of carcinoma of the breast with extensive metastases in the marrow of many bones in which as a result of extensive myeloid metaplasia the spleen was enlarged (15 × 9 cm) In the blood there were 15 500 erythroblasts per c mm and many myelocytes

Difficulties in differential diagnosis may only arise in cases of osteomyelosclerosis and osteomyelofibrosis (see p 64)

(b) *Chronic Inflammatory Types* In association with inflammatory stimuli some immature blood cells can always be found in the spleen myelocytes usually being more numerous than erythroblasts while megakaryocytes are rather uncommon As compared with the leukæmias the increase of myelocytes and erythroblasts is relatively small the former varying between 0 1 and 1 7 per cent and the latter between 0–2 per cent Such changes are much commoner in definitely chronic cases in which occasionally numbers as high as 9–15 per cent are found

Occasionally the splenomegaly and the appearance of immature cells in the blood may lead to confusion with leukæmia as in the following case —

H J aged 65 male
Had suffered from polyarthritis for 10 years In 1945 the spleen was found to extend to the navel In March 1943 in Professor Rossier s

vein with a persistent hard swelling of the spleen extending over *many years and repeated gastro intestinal bleeding* In the absence of any signs of liver disease Operation did not reveal any phlebitis in the splenic hilum but the greatly dilated veins and the numerous collaterals to the superior vena cava, together with the absence of signs *of portal stasis indicate that there was a mechanical obstruction* somewhere in the area drained by the splenic vein

During the first splenic puncture blood ran into the syringe no doubt as a result of congestion and it was only when a second puncture was performed with a *minimum* of suction that useful films were obtained and from these a splenogram was made and for comparison a second one was made from films obtained from the spleen at operation (Table 8)

The only change is slight increase of pulp cells such as has been found in all chronic inflammatory splenomegalies The plasmacytoid reticulum cells, staff forms and monocytes are not increased and an inflammatory condition can therefore be excluded with certainty this was later confirmed by histological examination In films from the spleen removed at operation there are rather more lymphocytes and fewer neutrophiles probably because blood had run out of the dilated sinuses

In another unusual case thrombosis of the portal and splenic veins had spread from a varicose dilatation in the liver The splenogram was normal except for a slight increase of neutrophiles probably due to admixture of blood on account of stasis in the spleen A third case also presented a normal splenogram

It seems clear therefore that a normal splenogram associated with well marked splenomegaly and the typical picture of splenopathic inhibition of the marrow strongly suggests splenomegalic cirrhosis or if the clinical features are consistent a chronic splenic or portal vein thrombosis Perhaps raised lymphocyte values of 80-85 per cent are rather more in favour of splenomegalic cirrhosis The presence of a definite inflammatory reaction is of course suggestive of an inflammatory cirrhosis If a puncture reveals a distinct increase of plasmacytoid reticulum cells in the absence of signs of inflammation the evidence in favour of cirrhosis is fairly strong

III SPLENOMEGALY WITH ECTOPIC FOCI OF BLOOD FORMATION

In this section some indications of the differential significance of distinct increase of immature myeloid cells in spleen puncture will be given Further details will be found in the relative sections

It has already been said that a few myelocytes and less commonly a few erythroblasts may be found in spleen puncture in chronic inflammatory conditions In some circumstances compensatory or

myeloid leukæmia but the toxic granules of the neutrophiles and the practically normal number of eosinophiles were rather against this view Sternal puncture showed distinct immaturity of myelopoiesis but not sufficient to make a definite diagnosis of leukæmia It was not until spleen puncture was done that the problem was solved This showed a typical inflammatory picture with a high percentage of neutrophiles (31 per cent) distinct increase of plasmacytoid reticulum cells (3 per cent) and slight increase of myelocytes and erythroblasts The latter were not as numerous as in cases of myeloid leukæmia in which we have found myelocytes to be between 20 and 60 per cent and the erythroblasts between 1 and 35 per cent except in erythro leukæmias where they may be as high as 59 per cent The low lymphocyte value (57 per cent) in the present case excluded aleukæmic lymphatic leukæmia as the cause of the great splenomegaly

Autopsy confirmed the diagnosis of a chronic inflammatory splenomegaly Probably this was an example of the rare splenic enlargement that may accompany chronic rheumatic polyarthritis (Veil (2)) which probably belongs to the group known as Still s disease The carcinoma of the œsophagus was not related to the enlarged spleen which was already noticeable long before any signs of the neoplasm developed

(c) *Leukæmic Types* The leukæmias are even quantitatively distinguishable by spleen puncture because of the much greater numbers of immature cells thus myelocytes in our observations varied between 8 per cent after arsenic or X ray treatment up to 60 per cent (Table 22) in a fully developed stage There are of course morphological abnormalities in the leukæmic cells (see p 130)

(2) *Predominantly Erythroblastic Types* The number of erythroblasts in spleen puncture is highest in four conditions viz hæmolytic anæmias 0 2–5 8 per cent pernicious anæmia during relapse up to 13 per cent myelosclerosis and osteosclerosis (5–20 per cent) leukæmias (1–35 per cent) especially the so called erythro leukæmias (up to 59 per cent in one of our cases)

(3) *Cases with Numerous Megakaryocytes* We found occasional megakaryocytes in chronic inflammatory states *e g* in spleen puncture of a case of undulant fever in which hæmolytic anæmia occurred Presumably the combination of the two morbid conditions were the cause of the extensive extramedullary blood formation In the foci erythroblasts were numerous (9 3 per cent) and myelocytes were found in each film

In inflammatory splenomegalic cirrhosis (see p 56) megakaryocytes were also numerous and were associated with up to 15 per cent erythroblasts

Megakaryocytes may be scanty or numerous in the leukæmias and also in osteosclerosis Lang has called attention to their pre

Clinic persistent leucocytosis of 12 000 to 14 000, with a few myelocytes was discovered The blood picture on March 19th, 1943 was red corpuscles 3 700 000 leucocytes 13 900 half mature myelocytes 1 5 per cent staff forms 6 5 per cent segmented polymorphs 66 5 per cent eosinophiles 2 per cent basophiles 3 5 per cent monocytes 4 per cent lymphocytes 16 0 per cent platelets 65,000 per c mm

The granules of the neutrophiles were rather larger than normal and *the nuclei of some of these cells were pyknotic A year later after a* period in which dysphagia had become troublesome he was admitted to the Clinic (May 1944)

There was distinct cachexia the spleen was 15 × 11 cm easily palpable and hard the liver and lymphatic glands not enlarged Red corpuscles 4 800 000 per c mm hæmoglobin 99 per cent , colour index 1 03 reticulocytes 2 1 leucocytes 29 700 mature myelocytes 0 5 staff forms 19 segmented polymorphs 70 eosinophiles 1 5 basophiles + monocytes 5 5 lymphocytes 3 5 platelets 267 000 The neutrophile *granules were rather coarse and tended to show slight basophilia Sternal* puncture showed erythroblasts 46 immature myelocytes 5 half mature myelocytes 9 mature myelocytes 3 7 metamyelocytes 1 0 *staff forms 39* segmented polymorphs 17 7 eosinophiles *2 3* monocytes 0 3 lymphocytes 23 0 The splenogram is shown in Table 9a

Radiography revealed the presence of a carcinoma of the œsophagus Death occurred on May 25th 1944

The autopsy showed advanced chronic inflammatory splenomegaly the organ weighing 850 g and measuring 23 × 12 cm Histologically *the trabeculæ were thickened the follicles very small and the pulp* extremely cellular with many pulp cells Hæmosiderin macrophage lymphocytes many neutrophiles a few eosinophiles and an occasional myelocyte were present Plasma cells were scanty The liver showed slight polymorph infiltration of Glisson s capsule but no round cell infiltration The carcinoma of the œsophagus was confirmed but no metastases were found

TABLE 9a

Puncture	1	2	Puncture	1	2
Hæmogram			Erythroblasts polychromatic	1 4	0 7
Leucocytes (in 1 000s)	29 7	—	Erythroblasts orthochromatic	0 2	0 4
Myelocytes	½	—	Erythroblasts Total	1 8	1 1
Neutrophiles staffs	19	—	Myelocytes immature	—	0 1
Neutrophiles segmented	70	—	Myelocytes half mature	0 1	0 4
Eosinophiles	½	—	Myelocytes metamyelocytes	0 2	0 3
Basophiles		—	Myelocytes Total	0 3	0 8
Monocytes	5½	—	Neutrophiles staffs	7 2	6 7
Lymphocytes	3½	—	*Neutrophiles segmented*	23 9	24 3
			Eosinophiles	0 1	0 1
			Basophiles	0 2	0 4
Splenogram			Monocytes	4 0	4 7
Macrophages	0 9	0 9	Lymphoblasts	0 1	0 1
Plasmacyt retic cells	3 0	2 9	Lymphocytes small	54 0	51 2
Pulp cells	0 6	0 2	Lymphocytes large	3 5	6 5
Tissue mast cells	0 1	—	Lymphocytes Total	57 6	57 8
Erythroblasts basophilic	0 2	—	Plasma cells	0 3	0 1

The large spleen and the persistently high leucocyte count with some myelocytes naturally first suggested a diagnosis of chronic

process can lead to osteosclerosis The typical overgrowth of the marrow may pass over into hypoplasia perhaps on account of the splenic hyperplasia when the sclerosis would be secondary to atrophy of the marrow On the other hand it might be supposed that the stimulus that causes the proliferation in chronic leukæmia may equally affect the related osteoblasts

Secondary sclerosis may occur in *aplastic anæmia* (Bock Wintrobe) Possibly some of these cases are due to a genuine chronic myelitis as Rohr has suggested Certainly similar pictures may occur as a result of damage to the marrow by chemical or physical agents *e g* fluorides strontium radio active substances gold etc (Bock)

The blood picture in osteosclerotic anæmia does not show much reduction of red corpuscles or hæmoglobin until quite late The number of granulocytes is usually slightly increased except in the type that develops secondary to aplastic anæmia then the lymphocytes are relatively increased There are always some erythroblasts and myelocytes both of which may b scanty or extremely numerous (Table 9b) In Muller s case which was proved by autopsy death followed irradiation of the spleen (which clearly indicates that it was not a case of leucosis) There were 15 100 leucocytes with 27 5 per cent of myelocytes and 16 5 per cent of lymphocytes while there were 4 erythroblasts per 100 white cells

The spleen is often very considerably enlarged and is fairly hard Extramedullary myelopoiesis is well marked with many erythro blasts myelocytes and megakaryocytes but unlike the condition in myeloid leukæmia the lymphatic follicles are well preserved (Fell Assman) Undoubtedly some cases belonging to this group have been published as megakaryocytic or aleukæmic chronic leucosis

No previous reports on splenograms of such cases are to be found Dr Sandkuhler of the University Medical Clinic Heidelberg and Professor Waldenstrom of the Medical Clinic Uppsala have kindly supplied us with the following spleen punctures on the typical cases of osteosclerotic anæmia (Table 9b) Case 1 which will be published in more detail (Sandkuhler) was one of a woman aged 50 who has suffered from abdominal distension for 13 years On admission to the Clinic her spleen extended into the pelvis The blood picture showed anæmia and with up to 44 per cent of immature myeloid cells and about 12 erythroblasts per 100 leucocytes The bones showed the typical radiographic changes and numerous attempts at marrow puncture failed to supply any marrow Even trephining of the upper end of the tibia was unsuccessful

The second case (Professor Waldenstrom) is probably one of myelosclerosis details of which have been published elsewhere (Moeschlin 25)

The patient a woman of 60 was admitted to hospital in 1937 with arthrosis of the hip and mild anæmia (72 per cent hæmoglobin 5 200

sence in polycythæmia and they are also present in splenic foci of blood formation in conditions in which the marrow space is diminished e g osteosclerosis (Schwartz) carcinomatosis of the marrow (personal observations) and in portal or splenic vein thrombosis (Bonner 1884 Eppinger 1920 Klemper 1928)

In films from the spleen of 2 cases of severe thrombocytopenia (*removal by operation*) *megakaryocytes were fairly plentiful In* both cases the cause was probably of an infective or toxic nature In the one case there was nephrosclerosis and in the other tuberculosis of the spleen and liver (p 77 and Fig 28)

IV OSTEOSCLEROTIC AND MYELOSCLEROTIC ANÆMIAS

The various conditions that lead to osteosclerotic anæmia (*myelosclerosis leuco erythroblastic anæmia etc) have at least one* feature in common diminution of the medullary space by osteosclerosis or myelofibrosis Clinically the spleen may become enlarged as a result of compensatory development of hæmopoietic foci in it while a similar change may occur in some lymphatic glands Immature myeloid cells appear in the blood but in many cases splenic hæmopoiesis fully compensates for the reduction of the marrow sometimes for years

Marble bone disease, an inherited malady which manifests itself in *childhood* belongs in this group (*vide* Naegeli Lang *Heilmeyer* Rosenthal), but there is a number of apparently allied but ill defined conditions

There are examples of primary increase of bony trabeculæ (diffuse or focal spongiosclerosis) and even of the compact bone but secondary sclerosis is also recognised Thus fibrosis of the marrow may occur in the leukæmias and aplastic anæmia Our view is that primary osteosclerosis if it exists is very rare as compared with the secondary type

Tischendorf and Naumann (4) did not find any examples in their series of leukæmias but we have seen 3 cases (3 typical chronic myeloid leukæmias and 1 erythro leukæmia) In these marrow puncture in the early stages revealed a very cellular marrow, while 2 or 3 years later puncture of the marrow of various bones failed to supply any myeloid tissue The bones also presented the radiographic signs of increased density and the unusual hardness was very noticeable when punctures were attempted Storti s cases manifested similar features and Bernard speaks of osteosclerose post leucosique as a late occurrence in some leukæmias It is quite likely that many published cases of osteosclerotic anæmia were really of this type

It is particularly interesting that an old standing leukæmic

leucocytes with a normal differential count) In 1943 she developed a normochromic anæmia with hæmoglobin 55 per cent leucocytes 5 200 premyelocytes 11 5 per cent myelocytes 7 per cent metamyelocytes 9 per cent and 25 erythroblasts per 100 leucocytes The spleen was distinctly enlarged (14/18 cm) and a number of bone punctures failed to supply any marrow In 1948 the hæmoglobin had fallen to 38 per cent and spleen puncture gave the results shown in Table 9b (Case 2) while sternal puncture only revealed a very hypocellular and œdematous jelly like marrow

Case 3 (Table 9b) a typical myelofibrosis will be published in detail elsewhere

Published reports on the pathological anatomy of the spleen in osteosclerosis and myelosclerosis show that predominance of lymphocytes in spleen puncture corresponds with persistence of the lymph follicles (see the cases of Reich and Rumsey Jackson Parker and Lemon etc)

There are a number of points of interest in these splenograms Thus if similar findings occur in other cases it ought in future to be possible to separate osteosclerotic anæmia from the secondary osteosclerosis of chronic myeloid leukæmia In splenograms of untreated cases of the latter disease the granulocytes were always between 75 and 97 per cent and even in erythro leukæmias if the erythroblasts and granulocytes are taken together the figures are similar while there is also distinct reduction of lymphocytes (1 5–20 per cent) Treatment with arsenic X rays urethane etc naturally changes these relationships considerably inasmuch as the immature cells diminish and the mature ones increase while the lymphocytes approximately double themselves (Table 22 cases 5 6 8)

In osteosclerotic anæmia on the other hand the percentage of lymphocytes is still high (up to 60 per cent) with a relatively small number of immature cells (myelocytes erythroblasts and occasional megakaryocytes) amounting to about 25 35 per cent *Thus in spite of the intense extramedullary blood formation the spleen still retains much of its original lymphadenoid structure whereas in the leukæmias this is totally obliterated and the tissue much more closely resembles bone marrow*

In most cases of chronic myeloid leukæmia the immature granulocytes also present signs of disturbance of development in the nuclei and cytoplasm whereas such changes are not found in the non leukæmic forms of extramedullary blood formation The occurrence of megakaryocytes in spleen puncture is not pathognomonic of osteosclerosis as they can also be numerous in cases of leukæmia (Figs 46 and 49)

A brief note on a case of *osteosclerotic leucosis* (see Case 4 Fig 26 *b*) in which we were able to perform a spleen puncture by the courtesy of Professor Waldenstrom will be given here (for further details see Moeschlin 25)

TABLE 9b

	Total leucocytes in the blood	Plasmacytoid reticulum cells	Splenogram: Erythroblasts			Myeloblasts	Myelocytes				Neutrophiles		Monocytes	Lymphocytes	Megakaryocytes
			Basophilic	Polychromatic	Orthochromatic		Immature	Half mature	Mature	Meta	Staff	Segmented			
Case 1 50 ♀			—	4 3	1 0		0 7	2 7	9 3	10 3	7 3	3 3			
	5 600	0 3	5 3			—	23 0				10 6		1 7	59 0	+
Case 2 60 ♀			6 0	12 2	1 2		—	4 0	4 0	7 0	3 6	0 8			
	5 200	0 6	19 4			—	15 0				4 4		—	60 4	+
Case 3 48 ♀			0 2	6 6	8 2		0 6	1 6	3 0	4 0	8 4	10 8			
	6 800	0 6	17 0			—	9 2				19 [illegible]		—	51 4	+
Case 4 37 ♂			2 0	13 8	0 4		0 8	6 2	13 4	21 6	7 8	3 0			
	[illegible]400	0 2	16 2			7 4	42 0				10 8		—	19 4	—

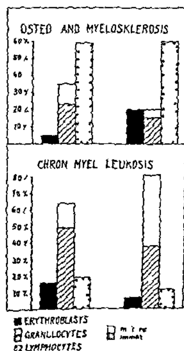


FIG [illegible]6 b *Comparison of the splenogram in compensatory extra medullary myeloid metaplasia of the spleen in osteo- and myeloscleroses with the spleen in chronic untreated myeloid leukæmia* In the former there are still 50–60 per cent of lymphocytes (Cases 1 and 2 above) *while in the latter there are only 5–25 per cent of lymphocytes* Case 3 was a severe osteosclerotic leucosis with a terminal para myeloblastic exacerbation *while Case 4 was an uncomplicated chronic* leucoses

In compensatory extra medullary myelopoiesis *in the spleen the fundamental* lymphatic structure of the organ remains more or less intact whereas in neoplastic *leukæmic infiltration this disappears completely*

Another point should be mentioned here In some cases of myelofibrosis we may be dealing with the subject of a toxic (gold etc) or inflammatory allergic damage to the marrow

Experience has shown that in such aplastic anæmias there is as a rule no extramedullary myelopoiesis Probably this is due to damage affecting the whole of the blood forming system so that the reversion of reticulum cells to erythropoiesis and myelopoiesis is inhibited also in the spleen

In view of the practical therapeutic importance of a careful clarification of the hereby only mentioned question of osteosclerotic anæmia we beg all observers to publish such cases in detail (with hæmogram histological findings of bone trepanation splenogram and X ray films of the bones) We hope that the collecting of more detailed observations will give us a clearer picture of this still problematic disease

V HÆMOLYTIC ANÆMIAS

The spleen may become considerably enlarged in hæmolytic anæmias in one of our cases of familial congenital icterus the spleen weighed 1 7 kg On the other hand in many cases of the so-called acquired form the hard spleen is only just palpable or only enlarged to percussion We have been able to investigate spleen punctures from 7 cases of hæmolytic anæmia in one this was combined with cirrhosis and in another with infection by *B Bang* The splenograms are shown in Table 10a In every case there was increase of pigment macrophages and a few polychromatic or oxyphilic erythroblasts were always present The highest values of these was in the case associated with cirrhosis (5 8 per cent) and in the one associated with Bang infection (9 3 per cent) Most of the pigment macrophages contained hæmosiderin in the form of a golden brown pigment which gave a prussian blue reaction In only one of the uncomplicated cases were the plasmacytoid reticulum cells increased (2 7 per cent) and also in the one associated with cirrhosis (2 4 per cent) The pulp cells were only increased in the case of Bang infection but often showed early deposition of hæmosiderin the lymphocytes values were never diminished

According to Lubarsch Gripwall and Heilmeyer there are no constant pathological findings Lubarsch mentions that the amount of hæmosiderin may be small or extremely great Gripwall using rapid fixation of spleens removed at operation found great dilatation of the sinuses the lining cells of which projected into the lumen The lymph follicles were small and scanty

Spleen puncture has not thrown any light on the genesis of the various forms of hæmolytic anæmia The one feature common to all types is increased blood destruction whether this be due to a

Case 4 was that of a man of 37 in whom an enlarged spleen and increased number of leucocytes (22 700 with 18 5 immature granulocytes) was first detected in 1945 The condition improved after irradiation of the spleen but in 1946 a hæmorrhagic diathesis developed X rays revealed a severe and progressive osteosclerosis in the whole skeleton In spite of repeated transfusions the general condition deteriorated and towards the end of life pathological micromyeloblasts were found in the blood and in spleen puncture (see Table 9b Case 3)

Spleen puncture in this case showed only 19 4 per cent of lymphocytes with 76 4 per cent erythroblasts and granulocytes of which about 7 4 per cent were small paramyeloblasts This was very unlike the other three cases

The terminal condition *viz* the appearance of pathological paramyeloblasts (micromyeloblasts) conclusively indicated that the disease was primary a leucosis

The presence of the severe osteosclerosis which was confirmed at autopsy may be secondary in the sense used by Bernard (osteo sclerose post leucosique) but on the other hand it may be that this may be an example of a condition suggested by Rohr *i e* a leucosis in which the involvement of the bones as well as of the marrow is attributed to a primary degeneration of the blood forming and bone forming mesenchyme

Rohr s opinion that the giant cells found in the spleen in these cases and in other leukæmic infiltrations are not megakaryo cytes was not confirmed by the appearances in our films These cells were of the typical transitional forms between immature and mature megakaryocytes

Spleen puncture permits such osteomyelosclerotic leucoses to be differentiated from osteosclerosis in which splenomegaly is due to a compensatory myeloid metaplasia In osteosclerosis due to leukæmia one finds as in the above case that in the spleen there is a striking reduction of the number of lymphocytes to 10–25 *per cent whereas in the compensatory type of splenomegaly the lymphocytes are rela tively high viz* 50–60 *per cent*

It appears therefore that in doubtful cases (of course in which there has been no treatment) relatively high lymphocyte values (over 50 *per cent) and relatively low myelocyte values* (20–30 *per cent) strongly suggest a true osteosclerosis or myelofibrosis rather than a genuine leukæmia*

It must be emphasised that in such cases removal of the spleen or exposure to X rays is strongly contra indicated because the com pensatory site of blood formation would be destroyed Even smal irradiations can prove fatal thus Hecht Johansen (1944) saw death occur as a result of agranulocytosis after administration of only 600 r in one of these cases while in Muller s case (1946) death occurred with progressive anæmia leucopenia and thrombo cytopenia

spleen appears to have shown that the spherocytosis is itself a cause of increased blood destruction because on account of their shape these corpuscles are not able to pass through the delicate reticulum of the pulp and so become separated from the plasma It is of course possible that the destruction of red corpuscles in some cases of hæmolytic anæmia may be due to direct phagocytosis in the spleen This factor is often stressed by pathologists (Hartmann) but in spite of careful search in our cases of hæmolytic anæmia we have never seen phagocytosis of red corpuscles by reticulo-endothelial cells It must be admitted however that we have not had the oppor tunity of performing a spleen puncture in the very early stage of a hæmolytic crisis Except in a case of *B Bang* but without any hæmolytic anæmia we saw one reticulum cell with phagocytosis of erythrocytes Baumgartner working on experimental anæmias in guinea pigs was able to demonstrate such phagocytosis in red corpuscles in the liver and spleen It is therefore possible that a similar condition occurs in some human cases and Schubothe recently demonstrated these findings in a case of hæmolytic anæmia due to an excessive amount of agglutinins

Owren s view that the hæmolytic crises depend upon a hypo plastic phase of erythropoiesis is open to criticism It is possible that in such cases there has been an inhibition of erythropoiesis as a result of kidney damage due to increased hæmolysis (augmented urea in all his cases) but as Rohr supposes the appearances are more likely to be due to intercurrent infection The latter could produce an arrest of the erythropoiesis followed by an excessive drop of the number of the erythrocytes in congenital hæmolytic anæmia due to the shorter life span of these cells (12–14 days) compared with a normal individual where an aplastic crisis (normal life span 120 days) causes a less drastic reaction of the erythro cyte number We have no doubt that in the absence of complica tions hæmolysis is the essential cause of the crises in acholuric jaundice and we have found some increase of erythroblasts in the sternal marrow at the onset of a crisis with further increases within 2 or 3 days if the cases were not complicated

This does by no means contradict the occurrence of aplastic crises Gasser could demonstrate typical aplastic reactions in different allergic conditions and we ourselves saw a permanent disappearance of all erythroblasts (erythroblastophtisis) in a case of aplastic anæmia (see p 73)

It is striking that the stimulus to erythropoiesis in hæmolytic anæmias leads to recurrence of blood formation in the spleen especially when hæmolysis is combined with an infective factor as in the following case —

P V male aged 27 whose brother and grandfather suffered from hæmolytic icterus had complained for some years of progressive fatigue

TABLE 10a

Case	Uncomplicated cases						
	1	2	3	4	5	6	7
Hæmogram							
Age sex	24 ♂	50 ♂	62 ♀	44 ♀	25 ♂	21 ♀	27 ♂
Osmotic resistance maximum	0 42	0 30	—	0 30	0 38	0 32	0 32
Osmotic resistance minimum	0 66	0 50	—	0 46	0 54	0 46	0 6[illegible]
Hæmoglobin (%)	96	59	—	72	85	70	52
Red corpuscles (in millions)	4 990	2 220	—	3 020	3 940	2 940	2 568
Leucocytes (in 1 000s)	8 4	3 2	—	27 8	4 8	3 4	5 0
Erythroblasts (per 200 leucocyt)	—	3	—	1	—	—	3
Reticulocytes (per 1 000)	—	92	—	159	31½	—	113
Neutrophiles staff	11½	22	—	42	28½	24	27
Neutrophiles segmented	59¼	25½	—	40½	34	30	19
Eosinophiles	1½	4	—	1	2	6½	½
Basophiles	—	1	—	½	1	2½	—
Monocytes	9	6½	—	3½	5½	14	8½
Lymphocytes	18	41	—	10½	29	23	44½
Splenogram							
Macrophages	0 3	1 8	2 3	+	—	0 6	0 5
Plasmacyt retic cells	—	2 4	2 7	+	0 2	0 7	0 4
Pulp cells	—	0 1	0 4	+	0 5	0 4	2 2
Erythroblasts basophilic	—	0 6	—	—	—	—	1 9
Erythroblasts polychromatic	—	3 9	0 1	—	—	—	5 8
Erythroblasts orthochromatic	—	1 3	0 1	—	0 1	—	1 6
Myelocytes mature	—	0 7	0 3	0 2	—	—	0 6
Myelocytes half mature	—	0 7	0 2	0 3	—	—	0 1
Myelocytes immature	—	0 2	—	—	—	—	—
Neutrophiles staff	3 3	5 0	1 1	0 8	3 0	6 5	7 6
Neutrophiles segmented	16 3	3 9	11 1	6 4	26 6	9 0	7 5
Eosinophiles	0 9	1 4	1 0	0 6	0 4	1 8	1 2
Basophiles	0 1	0 5	0 3	0 4	0 4	0 1	1 5
Monocytes	1 9	1 8	2 0	2 0	3 0	3 4	3 6
Lymphoblasts	—	0 1	—	0 6	—	0 8	—
Lymphocytes small young	7 5	4 2	2 6	10 0	3 9	7 5	2 3
Lymphocytes small old	61 5	68 5	74 2	66 3	58 7	56 3	61 3
Lymphocytes large young	3 0	0 7	0 3	6 1	0 6	6 3	0 3
Lymphocytes large old	4 9	0 6	0 9	5 8	2 6	5 7	1 5
Lymphocytes total	76 9	74 1	78 0	88 8	65 8	76 6	65 4
Plasmoblasts	—	—	—	0 1	—	0 5	—
Plasma cells half mature and mature	0 3	0 8	0 1	0 4	—	0 4	—

dımınıshed life span of the red corpuscles (as demonstrated in transfusion experiments by Dacie Ludin and Owren) in congenital familial cases or to formation of auto hæmolysıns auto-agglutinins or perhaps to lysolecithin (de Vries)

The investigations of Gripwall Dameshek and Schwartz indicate that the part played by the spleen is of the nature of an exaggeration of its normal function and that there is increased spherocytosis as a result of the action of hæmolysins while the corpuscles pass through the organ Probably the older and less resistant corpuscles are destroyed by these hæmolysins and then the reticulo endothelium of the spleen with that of other organs removes the debris

Bjorkmann, who has investigated the minute structure of the

foci in the spleen and this supports Rohr s view that these erythro blasts are not derived from the closed circulation of the bone marrow but enter the circulation from the open vascular system of the spleen and perhaps the liver

It is interesting that the infective stimulus evoked not only the formation of many erythroblasts and some myelocytes in the spleen but also the formation of considerable numbers of megakaryocytes This demonstrates clearly that such foci arise from metaplasia not from emigration from the bone marrow Megakaryocytes may be difficult or impossible to recognise in histological sections especially in the case of the young types in which the nucleus is not lobulated they are then likely to be classified as unspecific giant cells It is only in films that the morphological details can be detected The occasional myelocytes found in the peripheral blood especially during hæmolytic crises are also derived from extramedullary foci which are present in the spleen in most cases of hæmolytic anæmia

Numerous hæmosiderin macrophages were found in spleen puncture of a peculiar case of aplastic anæmia which was kept alive for 3 years by repeated blood transfusions although no erythroblasts could be found at all in the marrow (Table 10a case 6) This case was described in more detail by Moeschlin and Rohr (8) Heilmeyer (1942) and Begemann (1947) have reported similar cases and I have seen a similar one in which erythroblasts were absent from the marrow for at least 6 years (in the clinic of Professor Hansen in Oslo)

Such secondary hæmochromatosis must be due to the fact that the body is unable to make use of the excessive amount of iron derived from the transfused red corpuscles because erythropoiesis has ceased and the metal which cannot be eliminated is therefore gradually accumulated in the reticulo endothelium

Spleen puncture plays no part in the diagnosis of a typical case of hæmolytic anæmia the increased reticulocytes the raised serum bilirubin and the numerous erythroblasts in the sternal marrow enable a rapid diagnosis to be made In cases where the fragility of the red corpuscles is not increased and when during remission the only clinical sign is splenomegaly the presence of numerous hæmosiderin macrophages in spleen puncture may arouse a suspicion of latent hæmolytic anæmia especially if in the absence of inflam matory changes some erythroblasts are present

VI PERNICIOUS ANÆMIA

Splenomegaly is rarely pronounced in pernicious anæmia in adults but in younger patients may be quite considerable (Naegeli) Histologically the spleen shows well marked hæmosiderosis and numerous foci of blood formation (Lubarsch)

severe sweating and occasional slight jaundice On examination nutrition was good there was no fever a typical tower skull a high palate and a large hard smooth spleen (30 × 16 cm) The liver was also palpable The blood showed hæmoglobin 44 per cent red corpuscles 2 6 mill colour index 0 83 *spherocytes and polychromasia* The reticulocytes were 3 7 per cent but at a later examination 11 5 per cent Leucocytes 5 000 per c mm with 34 per cent lymphocytes The osmotic resistance was greatly diminished the maximum being 0 58 and the minimum 0 40 B S R 56 serum iron 120–170 bilirubin occasionally increased to 2 1

Sternal puncture showed 240 erythroblasts (basophiles 7 0 polychromatic 210 oxyphilic 23) to 100 white cells

Splenectomy was performed and the spleen was *examined in the* Pathological Institute at Zurich (Professor v Meyenburg) it weighed 1 7 kg and was 25 × 15 × 7 cm the capsule was smooth and on section the tissue was dark bluish red Histologically the follicles were fairly large there were epithelioid cells forming avascular nodules and in the outer zones of the follicles tuberculoid nodules were found in places The reticulum of the pulp cells was loose but with patchy thickenings and many foci of collagenous fibres and innumerable foci of epithelioid cells were present There were also infiltrations of the splenic veins The pulp was infiltrated with leucocytes plasma cells and occasional eosinophiles while in the vicinity of the vessels there were many hæmopoietic foci containing erythroblasts with small nuclei and a few *large basophilic erythroblasts*

Agglutination of *Bacillus Bang* was positive 1640 the hæmogram and splenogram are given in Table 10*a* Case 7

This was an example of typical familial hæmolytic anæmia with a strikingly large spleen in which histological examination showed the presence of typical Bang granulomata This case was described in collaboration with Loffler in an earlier publication on undulant fever

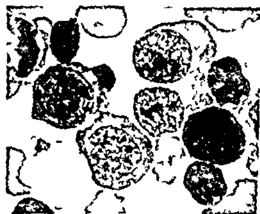


FIG 27 *a*

(*a*) Large basophilic erythroblasts (macroblasts) in a case of hæmolytic anæmia complicated by chronic inflammatory splenomegaly (Bang)

It must presumably be assumed that the hæmolytic anæmia was aggravated by the further increase of the splenomegaly due to the infection with *B Bang*

Films from the spleen showed a remarkable degree of erythropoiesis (9 3 per cent) with a considerable number of mitoses in the erythroblasts and many large macroblasts some being extremely young (Fig 27 *a*)

The presence of numerous erythroblasts in the peripheral blood (75 per c mm) was probably due to the numerous hæmopoietic

plasma stained by May Grunwald Giemsa corresponds to these bodies which probably are a special form of mitochondria (see Fig 27 c)

TABLE 10b

Myelogram	Splenogram										
			Megaloblasts			Myelocytes		Neutrophiles			
Megaloblasts	Plasmacyt retic cells	Pulp cells	Basophil	Polychrom	Oxyphil	Half mature	Mature	Staff forms	Segmented	Eosinophiles	Lymphocytes
			2 4	10 6	0 6	2 2	3 6	4 6	11 0		
57 0	2 4	0 6	13 6			5 8		15 6		3 4	53 8

A number of authors (Nagy 1924 Introzzi 1929 and Weil 1936) agree that megaloblasts as well as myelocytes are found in spleen films

Since the introduction of sternal puncture spleen puncture has ceased to be of diagnostic value in pernicious anæmia but it does demonstrate that in this disease there is a general disturbance of blood formation which leads to the development of such abnormal elements as megaloblasts even in extramedullary erythropoietic foci It also demonstrates that the development of megaloblasts does not depend upon some local peculiarity but only upon deficiency of a hæmopoietic factor and that in its absence megaloblasts can arise from the reticulum cells of the spleen

In our view these findings are also proof that megaloblasts in the circulating blood in pernicious anæmia are not derived from the bone marrow but from extramedullary foci mainly in the spleen

VII THROMBOCYTOPENIA WITH SPLENOMEGALY

It is recognised that the spleen plays a considerable part in the genesis of many forms of thrombocytopenia (Naegeli Rohr Heilmeyer) At least two processes are at work viz inhibition of maturation of megakaryocytes in the marrow probably as the result

In a man of 67 with typical pernicious anæmia (hæmoglobin 37 per cent and red corpuscles 1 2 mill) there were many megalocytes in the blood and a typical megaloblastic marrow, in spleen puncture up to 13 6 per cent typical large megaloblasts were found (Fig 27, *b*) Mitoses were fairly frequent and the number of megaloblasts in the spleen was greater than that in the peripheral blood (1 megaloblast per 100 leucocytes) so that there can be no doubt



FIG 27 *b c d* *Megaloblasts (14 per cent) in spleen puncture in pernicious anæmia*

(*b*) May Grunwald stain a mitosis on the left
(*c*) Round colourless gap in the otherwise basophil plasma of a young megaloblast (May Grunwald Giemsa)
(*d*) Phase contrast picture Here an agglomeration of brightly shining globules (Kugelhaufchen) can be seen (Zeiss phase contrast microscope 1 000)

that these abnormal cells can arise in the spleen and it was striking that they were on the whole more mature in the spleen than in the sternal marrow No obvious explanation is available but we have found a similar difference in the degree of maturation in chronic myeloid leukæmia

In early megaloblasts with the phase contrast microscopy (Moeschlin (23)) an agglomeration of brightly shining globules could be found (Kugelhaufchen) which could not be observed in other cells A round colourless gap in the otherwise basophil

plasma stained by May Grünwald Giemsa corresponds to these bodies which probably are a special form of mitochondria (see Fig 27 *c*)

TABLE 10b

Myelogram	Splenogram										
			Megaloblasts			Myelocytes		Neutrophiles			
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of a splenic hormone and also increased destruction of thrombocytes in the spleen itself Any disease associated with splenomegaly may therefore, be accompanied by thrombocytopenia and if this is *sufficiently severe with a hæmorrhagic diathesis Thrombocyto* penia of splenic origin has been observed in a very great variety of splenic diseases *e g* in such non infective enlargements as that of Gaucher s disease and splenomegalic cirrhosis and also in those of infective origin such as chronic malaria In a woman aged 44 (No 4 Table 10a) with a typical hæmolytic anæmia in this way we *observed as a great exception an extreme and fatal thrombocytopenia (10 000 platelets per c mm) although splenectomy was performed*

This case cannot be interpreted as an hormonal hypersplenism because the leucocytes were 7 800 per c mm and the reticulocytes 16 6 per cent It appears clear that in many such cases the splenic thrombocytopenia is mainly due to increased destruction of platelets by the spleen and that the increase of megakaryocytes in the marrow is a response to this increased utilisation of platelets

In chronic Werlhof s disease in which splenomegaly although constant is never great the chief defect is still unknown but dis turbance of the maturation of megakaryocytes appears to be one of the main factors The pathological conditions found in the spleen vary from case to case (Heilmeyer Schulten)

Then again in acute myeloblastic leukæmia with splenomegaly there is often severe thrombocytopenia but here the principal factor is not to be found in the spleen but in the leukæmic proliferation in the marrow which leads to disappearance of megakaryocytes

Spleen puncture is totally contra indicated in a case of hæmor rhagic diathesis associated with severe thrombocytopenia Diagnosis will have to depend upon careful analysis of the blood and sternal marrow Only if the platelets temporarily increase and other methods reveal no tendency to hæmorrhage can spleen puncture safely be used In the following case (Case 1) there was typical Werlhof s disease in a man of 35 in whom repeated blood transfusions suc ceeded in making splenectomy unnecessary Spleen puncture showed an almost normal splenogram (Table 11) in which there was however some increase of eosinophiles and plasmacytoid reticulum cells Platelets are as we have already mentioned more numerous in spleen punctures than in blood films The presence of plentiful platelets in spleen puncture in *Werlhof s disease obviously cannot be regarded as evidence of a thrombocytolytic activity of the* spleen In none of our 3 cases did we observe an increase of macro phages in fact they were rather fewer than in most other diseases (0 1–0 2 per cent) It seems very doubtful therefore whether the spleen in this case removed platelets from the blood by phagocytosis on the part of the reticulo endothelial cells When one remembers the extreme fragility of the platelets *it would seem unlikely that they*

would be removed in this manner and by analogy with red corpuscles by hæmolysin solution in the spleen appears to be more probable (Gripwall Dameshek and Schwartz) probably in addition to hormonal inhibition of the marrow (Naegeli)

TABLE 11

Case	1	2	3
	Morbus Werlhof	Toxi tuberc	Toxi allergic (nephro-sclerosis)
Age sex	35 ♂	38 ♂	30 ♀
Hæmogram			
Hæmoglobin (%)	—	10-	53
Red corpuscles (in millions)	—	5 888	2 616
Thrombocytes	—	½ ‰ = 2 944	0 ‰
Leucocytes	—	8 400	7 900
Myelocytes	—	—	1½
Neutrophiles	—	74½	73
Eosinophiles	—	6½	1½
Basophiles	—	1	½
Monocytes	—	10½	3
Lymphocytes	—	7½	20
Plasma cells	—	—	½
Splenogram			
Macrophages	0.1	0.2	0.1
Pla mıcyt retic cells	0.8	1.0	0.1
Pulp cells	—	—	0.3
Erythroblasts	—		7.7
Myelocytes	—	—	0.2
Neutrophiles staff	1.7	3.9	4.1
Neutrophiles segmented	21.9	11.8	4.1
Eosinophiles	4.7	1.2	0.4
Basophiles	1.1	0.3	0.1
Monocytes	1.3	3.6	3.6
Lymphoblasts	—	—	0.5
Lymphocytes young	1.0	0.9	3.3
Lymphocytes old	67.4	77.0	75.9
Lymphocytes total	68.4	77.9	79.7
Plasma cells	—	0.1	1.5
Megakaryocytes	—		0.1

We have observed 2 cases in which thrombocytopenia was probably due to toxins and in which splenectomy was performed

Case 2 A man aged 38 had suffered from repeated bleeding for 2 years The spleen was not enlarged the platelets were at first between 11 and 35 000 later 2–3 000 per c mm the blood picture was otherwise normal Sternal puncture showed great increase of megakaryocytes particularly of the immature forms but no other pathological changes No method of treatment produced a rise of platelets and the spleen was therefore removed Films made from it showed typical tuberculous epithelioid cells no myelocytes but fairly plentiful megakaryocytes (Table 11 Case 2) Histologically the spleen showed many calcified tubercles up to about 0.5 cm in diameter The platelets did not rise after operation and death occurred a week later

Autopsy revealed old calcified tuberculosis of the liver The cause of death was pneumonia probably associated with extensive bronchiectasis

In this case there was, almost certainly a toxi infective disturbance of the maturation of megakaryocytes due to tuberculosis of the spleen and liver and perhaps also to the chronic bronchiectasis It was not therefore, an example of *thrombocytopenia due to* hypersplenism Kellert Zorini Alessandri Reggiani and Lapp have published similar cases of thrombocytopenic purpura associated with splenic tuberculosis

The present observation shows that compensatory foci of mega karyocyte formation may develop in such cases a fact which had been noted previously by the pathologists Lang and Lubarsch The following observation is of special interest

Case 3 A woman aged 30 had an attack of polyarthritis in June 1934 and a month later presented signs of a severe hæmorrhagic diathesis In September she was admitted to the surgical clinic (Professor Clairmont) Red corpuscles 2 million hæmoglobin 33 per cent, platelets almost absent (about 2 000 per c mm) bleeding time more than 1 hour coagulation time 2 minutes reticulocytes 5 6 per cent leucocytes 3 700 per c mm The spleen was not palpable but was enlarged to percussion Only blood was obtained when sternal puncture was performed On the advice of Professor Naegeli splenectomy was per formed on September 14th The histological diagnosis was that of a myeloid reaction associated with thrombocytopenia

Following operation there was pyrexia and a relapse of the joints condition there was no rise of platelets and a tentative diagnosis of peliosis rheumatica was made For this reason large doses of pyramidon were given The temperature fell and the platelets slowly rose until they reached 120 800 per c mm on October 21st In January 1936 the joints were again troublesome and the platelets had fallen to 17 000 per c mm Mitral disease was now present (red corpuscles 2 800 000 per c mm hæmoglobin 74 per cent leucocytes 6 600 the urine contained 0 15 per cent of albumin) A number of infected teeth were present These were removed and pyramidon was administered again the platelets did not rise and death occurred on May 20th as a result of uræmia

The autopsy showed verrucose endocarditis of the mitral valve associated with considerable nephrosclerosis The femoral marrow contained many megakaryocytes some of which had greatly lobulated nuclei indicative of inhibition of the maturation

This is an example of severe thrombocytopenia accompanying rheumatic infection and endocarditis with renal sclerosis It seems probable that all these processes depend upon sensitisation to certain bacterial toxins Unfortunately the condition of the marrow before death was not known but the post mortem findings in the femur and the changes in the blood picture can be interpreted as indicative of interference with maturation of megakaryocytes due to allergic and toxic cause

In peliosis rheumatica the platelets are not diminished (Heilmeyer) and can therefore be excluded as can also a hæmorrhagic diathesis due to capillary damage It is striking that splenectomy had no effect on the thrombocytopenia and that it was only when the temperature fell as a result of administering pyramidon that there

was a rise of thrombocytes It appears to us therefore that in this case the rheumatic process not hypersplenism was responsible for the thrombocytopenia There are other similar cases to be found in the literature in which chronic joint rheumatism has been accompanied by splenomegaly and thrombocytopenic purpura (Veil)

Spleen films (Case 3 Table 11) showed numerous megakaryocytes (about 1 per 1 000 cells) together with greatly increased extramedullary myelopoiesis which perhaps because of the frequent and severe hæmorrhages was predominantly erythropoietic This case is unique because even in chronic myeloid leukæmia we have

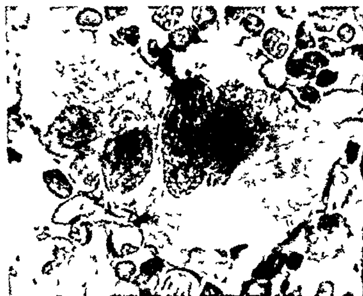


FIG 8 Megakaryocytes in spleen films from a case of thrombocytopenic purpura

never seen so many megakaryocytes (Fig 28) Certainly in this case the condition was not leukæmic the low percentage of myeloid cells (only 0 2 per cent of myelocytes with increase of erythroblasts to 7 7 per cent) and somewhat raised lymphocytes excluded this possibility while the excess of lymphatic plasma cells indicates the chronic inflammatory nature of the condition

B SPLENOMEGALIES OF INFLAMMATORY ORIGIN

VIII ACUTE AND SUBACUTE SPLENOMEGALIES

Here are included those enlargements of the spleen that occur in some acute infections and which may pass over into a more chronic stage

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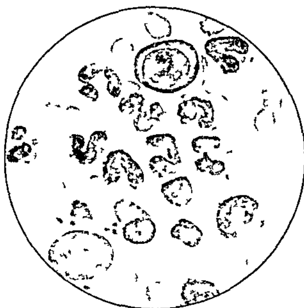


FIG 29 Acute inflammatory spleen Numerous toxic neutrophiles occasional myelocytes and macrophages and one young lymphati plasma cell (Painting by Mrs Bollinger Schudel)

The most striking feature (Table 12) was the very great increase of neutrophiles (72 per cent) with only 19 per cent of lymphocytes with a marked shift to the left and toxic changes in the former Plasmacytoid reticulum cells were also increased an indication that the inflammation was of long duration while of course also

TABLE 12

	Hæmogram						Splenogram												
No of case	Leucocytes (in 1 000s)	Myelocytes	Neutrophiles staff	Neutrophiles segmented	Monocytes	Lymphocytes	Macrophages	Plasmacyt retic cells	Pulp cells	Myelocytes	Neutrophiles staff	Neutrophiles segmented	Eosinophiles	Monocytes	Lymphoblasts	Lymphocytes young	Lymphocytes old	Total lymphocytes	Plasma cells
1	7 3	½	76½	15	1	7	0 3	3 2	0 7	1 7	63 1 (72 3)	9 2	0 2	1 2	0 1	1 9	17 2	19 2	1 2
2	7 4	—	39	44½	9½	6½	0 6	0 [illegible]	0 5	0 7	24 6 (59 5)	34 9	0 8	8 2	—	5 [illegible]	23 7	28 9	0 5

The pathology and histology of acute splenic swelling (Lubarsch) is well known We will only mention the congestion of the organ the leucocytic infiltration of the pulp the hyperplasia of the reticulo endothelium sometimes accompanied by deposition of hæmosiderin and the occasional development of hæmopoietic foci (Hirschfeld)

The predominance of granulocytes in most acute infective diseases due to bacteria has recently been investigated (Ehrich *et al*) It is assumed that in virtue of the presence of their enzymes corpuscular antigens are split into soluble antigens and so prepare for the anti body building cells (plasmacytoid reticulum cells) In this way the neutrophiles contain mainly protein and carbohydrate splitting enzymes and the macrophages fat splitting ferments This functional difference explains the conversion of the acute inflammatory picture (neutrophile reaction) into the chronic inflammatory one (monocytic plasma cell reaction)

Our observations have led us to divide acute inflammatory splenomegalies into two groups those with a predominantly neutrophile response (bacterial infections) and those with a mainly lymphocytic one (virus infections)

1 NEUTROPHILE REACTIONS

The type occurs most commonly in acute septic diseases We have not punctured the spleen in the early stages of such maladies because of the friability of the organ at this time If however the disease persists and other methods have not made the diagnosis clear spleen puncture can safely be performed if our technique is carefully followed especially as a very thin needle can be used because of the cellularity of the pulp

We have never punctured a typhoid spleen but Vidal (1890) and Hayashihava (1909) often did so in order to obtain cultures and indeed the latter reported on 109 cases

The main diagnostic value of spleen puncture in septic diseases is the demonstration of the causative bacteria We have succeeded by this means after several blood cultures were negative In other cases of pyrexia the explanation may be given by the finding of Dorothy Reed or tuberculous epithelioid cells (see pp 104 and 117)

A woman of 36 had had high fever for 2 months with greatly increased erythrocyte sedimentation and leucocytes of 15–20 000 with much toxic change in the neutrophile granules The spleen was just palpable All cultural and agglutination tests were negative and after 6 weeks illness spleen puncture revealed the splenogram shown in Table 12 Case 1 No growth of pyococci was obtained and *unfortunately*, no attempts were made to grow tubercle bacilli but a few weeks later there was growth from a bilateral pleural effusion The temperature fell after 2 months and recovery ensued from what must have been a severe hæmic spread of tuberculosis

mononucleosis in an attempt to gain further information as to the origin of the characteristic cells In 2 cases there was splenomegaly but not enlargement of lymph nodes while in another only the spleen and hilum glands were enlarged As Chevallier has pointed out such purely splenomegalic cases are by no means uncommon Occasionally the enlarged spleen may lead to so great a degree of inhibition of the marrow that the disease runs the course of agranu



FIG 30 Spleen puncture in infective mononucleosis

(a) Numerous lymphoid monoblasts (x) and mature lymphoid monocytes (xx)—glandular fever cells
(b) Mitoses in such cells
( ) Abnormal (tripolar) mitosis in a young glandular fever cell

locytosis (Tidy and Heilmeyer (4)) we have seen one case in which the granulocytes numbered only 500 per c mm The rare thrombocytopenic cases (Bernstein Lloyd) are probably explicable in the same way

In an earlier investigation (Moeschlin (5)) we found up to 11 per cent of large reticulo endothelial cells in gland punctures at the height of the disease and among these there were many mitoses from which all transitions to the characteristic glandular fever

proving that spleen tissue not merely blood was present in the films Myelocytes (1 7 per cent) were numerous and must have been derived from ectopic hæmopoietic foci a further sign of the chronicity of the inflammation No epithelioid giant cells or any other indication of tuberculous infection could be found, so that it is clear that a severe hæmatogenous tuberculosis in its early stages produces changes in the spleen similar to those of any other septic infection

The second example was a man of 63 who for 3 weeks, had suffered from cholangitis with great splenomegaly The splenogram (Case 2 Table 12) showed marked neutrophilia associated with increase of monocytes (Fig 32)

2 LYMPHATIC REACTIONS

Distinct splenomegaly can be detected in a number of diseases that are accompanied by a lymphatic response in the blood (lymphotropic virus diseases) These conditions excluding lymphatic leukæmia which is not to be considered as being infective will now be discussed in the light of spleen puncture

(a) Infectious Mononucleosis

Nowadays this term is used to indicate a clinical picture which includes what used to be called lymphocytic angina 'monocytic angina lymphoid cell angina and Pfeiffer s glandular fever (see Lehndorff and Schwarz) The term mononucleosis is to be preferred to glandular fever because lymphadenopathy does not occur in all cases especially in adults

Heilmeyer regards all the cases as being of the same ætiology basing his view on the bacteriological and experimental work of Bland (1931) Wising (1939) and Berghe (1939) Schulten and Josephs however held that some diseases with a mononuclear blood picture are caused by other agents Thus Josephs in America has described a condition resembling influenza in which extensive swelling of lymph nodes and a characteristic mononuclear blood picture occur during convalescence Haberfeld also in South America has described a similar infectious condition which is conveyed by the bites of certain ticks Hittmair observed an epidemic of thrush with increase of lymphocytes including some atypical cells in the blood We have reported lymphatic monocytoid pictures in virus pneumonia (Moeschlin (10)) a few similar cells together with typical lymphatic plasma cells are fairly regularly seen in infective hepatitis and rubella Lymphocytosis (up to 70–80 per cent) mainly due to increase of old lymphocytes is seen in lymphocytosis acuta (see p 91) It seems probable therefore that further research especially on viruses may distinguish other groups

We have punctured enlarged spleens in 8 cases of infectious

mononucleosis in an attempt to gain further information as to the origin of the characteristic cells. In 2 cases there was splenomegaly but not enlargement of lymph nodes, while in another only the spleen and hilum glands were enlarged. As Chevallier has pointed out, such purely splenomegalic cases are by no means uncommon. Occasionally the enlarged spleen may lead to so great a degree of inhibition of the marrow that the disease runs the course of agranu-

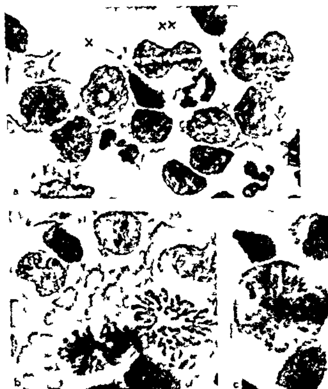


FIG. 30. Spleen puncture in infective mononucleosis.
(a) Numerous lymphoid monoblasts (x) and mature lymphoid monocytes (xx)—glandular fever cells.
(b) Mitoses in such cells.
(c) Abnormal (tripolar) mitosis in a young glandular fever cell.

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In an earlier investigation (Moeschlin (5)) we found up to 11 per cent. of large reticulo-endothelial cells in gland punctures at the height of the disease, and among these there were many mitoses from which all transitions to the characteristic "glandular fever

cells ' could be discovered We called these elements lymphatic monoblasts ' and the more mature cells arising from them lymphatic monocytes

First we will describe our observations on the structure of these reticulo endothelial cells as shown in our gland and spleen punctures, but as not all the cells present monocytoid indentation of the nucleus and as the number of mature forms with lobed nuclei varies from case to case we shall here only distinguish lymphoid mono

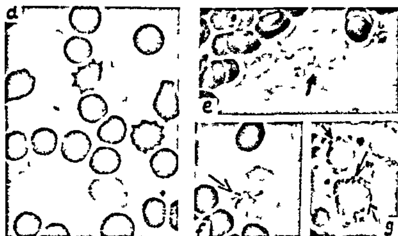


Fig 30 *d e f g* *Phase contrast pictures*

(*d*) *Ordinary lymphocytes of blood* They show a fine greyish scanty granulation

(*e* and *f*) *Cells of infectious mononucleosis in blood* Contrary to the normal lymphocytes they show a relative coarse dark (black like) granulation which indicates a reticulo-endothelial origin They are differentiated clearly from normal monocytes which contain no dark granulation Fig *e* shows a mitosis of an infectious mononucleosis cell in blood

(*g*) *A phase contrast photograph of two small lymphoid reticulum cells* They show in the plasma a dark granulation arranged in droplets which is similar to the inclusions of plasmacytoid reticulum cells and the cells of myeloma These findings are perhaps in correlation with the production of certain globulins by these cells

blasts and among the mature cells lobed and unlobed lymphoid monocytes

The use of the word lymphoid rather than lymphatic is necessary because van Beck has shown that these cells are not formed only in the lymphatic system but also in the reticulo endothelium of the liver and perhaps of other organs These elements are definitely pathological the formation being evoked by certain specific stimuli

In the phase-contrast microscope these glandular fever cells show distinct coarse granules in the cytoplasm (Fig 30 *e* and *f*) which are absent in the usual blood monocytes thereby and by the negative glycogen reaction (Gibb and Stowell) these cells differ also physically and chemically from the normal blood monocytes

Structure of Lymphoid Monoblasts These are large rounded or oval cells (12-25 μ) The cytoplasm varies from pale to dark blue often being rather cloudy Azure granules are absent from the youngest types but a few may be seen in the more mature cells The nucleus which is round or oval occupies the greater part of the cell (14-20 μ) and may show early indentation In the youngest forms the chromatin shows a fairly close but coarse meshwork (Fig 30) in which lie 2 or 3 fairly large but rather indistinct nucleoli

Methyl green pyronin stains the cytoplasm red (pale or deep) and the nucleus greenish blue The peroxidase reaction is negative

The coarseness of the nuclear texture and the histo-chemical characters clearly distinguish these cells from the oxydase positive myeloid monocytes

Mitoses in monoblasts (Fig 30 *b*) show so close a resemblance to those in plasmoblasts that they cannot be distinguished from one another in films although perhaps their chromosomes are a little shorter and broader Occasionally atypical mitoses occur as in the case of the tripolar one shown in Fig 30 *c*

Lymphoid monoblasts can be distinguished from plasmoblasts by the closer and striate arrangement of the chromatin whereas that of the latter is quite delicate and evenly distributed Some authors (Glanzmann Leitner) refer to glandular fever cells as lymphoblasts but this is undoubtedly incorrect (Schwarz Downey Heilmeyer) because these are smaller never have deep blue cytoplasm and exhibit more delicate nuclear structure

Spleen punctures show that cytogenesis reaches its greatest intensity in the first week perhaps persisting into the second week and then diminishing rapidly (Table 13) The changes in the spleen and lymph nodes commence before those in the blood and disappear earlier At first mitoses in monoblasts are numerous in Case 3 spleen puncture on the 15th day of the illness showed 0 6 per cent i e, mitoses in 13 per cent of the total monoblasts In Cases 1 and 5 one third and a quarter respectively of the monoblasts were in mitosis

The characteristic feature of the spleen and lymph nodes at the height of the reaction is the occurrence of many immature and mitotic reticulo endothelial cells (lymphoid monoblasts) and all transitions to the more mature elements seen in the blood can be detected

The most immature elements are very scanty in the blood but even mitoses may be seen as we have observed in one case (also Schwarz Nyfeldt Angelini) The number of normal lymphocytes in the spleen and lymph nodes is somewhat decreased during the phase of reticulo endothelial irritation but during convalescence there is first increase of lymphocytes with broad cytoplasm and later of typical small ones

At the height of the illness plasma cells (1-5 per cent) identical with those seen in rubella are regularly present in the blood but it is incorrect to follow Waitz who would refer to all cells with well marked cytoplasmic basophilia or with distinct chromatin

spokes as plasma cells We assume that the morphological resemblances are due to the fact that both the lymphatic plasmoblast and the lymphoid monoblast have a common origin from the reticulo endothelium *i e* from the large lymphatic reticulum cell which forms the germinal centres in lymph nodes Even so as described above differences are detectable in the very immature forms

TABLE 13

Infective Mononucleosis

Case number	1	2	3	4	5	6	7
Day of disease	*6th*	*10th ?*	*15th*	*23th*	*24th*	*?*	*28th*
Hanganatziu Deicher reaction	+ 1 80	+ 1 40	++ 1 160	+++ 1 640	+− 1 160	−+ 1 160	2mal neg
Hæmogram							
Leucocytes	9 000	8 700	9 100	6 800	7 100	5 800	8 900
Neutrophiles staff	11½	15	14	27½	15	22	18½
Neutrophiles segmented	40	24	7	14	17	11	40½
Lymphocytes	67½	52	71	54	58½	53	29½
Plasma cells	1	1	3	½	—	¾	—
Splenogram							
Macrophages	0 3	+	+	+	—	0 2	—
Plasmacyt retic cells	1 1	2 0	1 1	0 2	1 8	2 8	0 6
Pulp cells	0 9	1 1	1 0	1 0	0 2	1 5	0 2
Erythroblasts	—	—	0 1	0 8	0 1	—	0 2
Myelocytes	0 3	0 8	0 1	0 2	—	0 1	0 1
Neutrophiles staff	8 4	4 8	7 4	7 6	3 0	12 1	5 3
Neutrophiles segmented	5 6	10 3	4 0	10 5	3 8	9 5	14 2
Eosinophiles mature	0 6	0 3	0 2	1 0	1 9	1 0	1 1
Basophiles mature	0 2	0 7	0 2	1 6	1 1	0 3	0 8
Monocytes	3 9	3 8	4 6	5 5	4 0	9 3	3 8
Lymphoblasts	—	0 1	—	—	—	—	—
Lymphocytes small young	3 0	5 8	2 5	4 5	5 8	4 5	2 8
Lymphocytes small old	59 3	44 2	42 4	29 4	62 7	41 1	65 0
Lymphocytes large young	0 9	1 6	0 1	0 1	1 1	0 8	0 8
Lymphocytes large old	3 3	15 4	5 9	9 5	4 5	5 3	2 8
Lymphocytes total	*66 5*	*47 1*	*50 9*	*43 5*	*74 1*	*51 7*	*71 4*
Lymphoid monoblasts (glandular fever cells)	1 2	2 6	4 5	0 2	1 6	0 4	0 2
Glandular fever cells unlobed	6 4	13 4	16 0	14 9	4 0	6 2	1 8
Glandular fever cells lobed	3 5	12 2	7 2	14 8	3 6	7 5	0 2
Total of lymphocytes and glandular fever cells	*77 6*	*75 1*	*78 6*	*71 4*	*83 3*	*60 8*	*73 6*
Plasmoblasts	—	0 1	0 1	—	0 1	—	—
Plasma cells half mature and *mature*	1 1	1 0	2 6	0 2	0 7	2 4	0 1

In spleen puncture at the height of the disease lymphatic plasma cells are only slightly increased (1 3 per cent) unlike the state of affairs in epidemic jaundice in which they are numerous

As in all inflammatory conditions of the spleen myelocytes (0 1–0 8 per cent) and a few polychromatic erythroblasts can be found Pulp cells are moderately increased in number (up to 1 5 per cent in Case 6) a sign of any inflammatory reaction The scanty neutrophiles show a distinct shift to the left Otherwise

apart from the occasional presence of tissue mast cells and isolated fat cells the splenogram presents no other peculiarities

Origin of Glandular Fever Cells in the Spleen Our earlier investigations on puncture of lymph nodes led us to accept the view that the essential feature was a reaction of the reticulo endothelial cells of the lymphatic follicles It is probable that the majority of the lymphoid monoblasts in spleen films arise from the reticulo endothelium of the follicles whereby in lymphatic tissue they may arise from the ' large lymphatic reticulum cells

Van Beck and Haex have shown clearly that the reticulo endothelial system of the liver also contributes to the glandular fever cells because in liver punctures they found great increase of reticulo endothelial elements with many mitoses Heilmeyer Rohr and Schleicher saw a similar reaction in the sternal marrow It seems likely that Heilmeyer s case of mononucleosis accompanied by symptoms of nephritis and also the occurrence of mononuclear reactions in the cerebro spinal fluid (Huber Gsell) indicate that the reticulo endothelium of the kidneys and meninges are involved in some cases the latter also in the occasional encephalitic type (Geliebter)

All these observations force us to the conclusion that infective mononucleosis is not characterised by reaction purely of the lymphatic system but by one of numerous parts of the reticulo endothelium which are involved according to the localisation of the unknown cause The disease therefore would have to be regarded as a secondary infective reactive reticulosis It is thus possible that in the spleen it is not only the reticulo endothelial cells of the lymphatic follicles but also those of the red pulp that are involved

This question cannot be decided on the basis of the older cases in the literature (Dubois Tremolhieres Haken) but the more recent reports of 8 cases with spontaneous rupture in the spleen (Smith 1946 Vaughan 1946) seem to confirm the present view Smith s case showed dense lymphoid infiltration of the spleen capsule and trabeculæ Darley found the red pulp completely infiltrated with lymphatic cells but he did not find any mitoses perhaps because the disease was past its maximum (17th day) In Ziegler s case there were lymphatic infiltrations in the liver kidneys spleen and lungs

The only reports of spleen puncture in infective mononucleosis was made by Sigon and Weil Sigon found a considerable increase of reticulo endothelial cells with many macrophages but we have not been able to confirm the latter finding Weil unfortunately does not mention the period of illness when he performed his puncture but at a time when the blood picture contained 49 per cent lymphocytes and monocytoid cells the splenogram showed 28 per cent typical lymphocytes 19 per cent young lymphocytes and 43 per cent monocytes with 0 5 per cent mitoses

Spleen puncture in infective mononucleosis is not really of

diagnostic value because a proper knowledge of the structural differences between leukæmic cells and glandular fever (broad plasma ?) should permit of differential diagnosis without difficulty It is only in cases with very well marked monocytoid forms that confusion can occur and then sternal puncture would rapidly settle the question

As a number of cases of spontaneous rupture of the spleen have now been reported we should strongly advise against spleen puncture in any case of infective mononucleosis in which the organ is tender

(b) Epidemic Hepatitis

Epidemic hepatitis is another of the virus diseases in which distinct lymphatic reaction occurs We are in a position to report on 3 cases in which the spleen was punctured in one of them twice (Table 14)

Between the 5th and 11th day of the illness the spleen showed an increase of large lymphatic plasma cells and of their precursors the half mature plasma cells and plasmoblasts (Fig 31 *a*) with many

TABLE 14

Hepatitis Epidemica

Case number	1	2	2	3
Day of disease	6th	9th	11th	14th
Hæmogram				
Total leucocytes	8 600	6 900	5 300	15 700
Neutrophiles staff	18½	21	10	25
Neutrophiles segmented	15½	48	53½	33
Lymphocytes	32	16½	19	27
Plasma cells	17½	½	1	1½
Splenogram				
Macrophages	+	+	0 2	—
Plasmacyt retic cells	0 6	1 1	0 6	3 1
Pulp cells	0 2	0 2	2 7	1 4
Erythroblasts	—	—	—	0 1
Myelocytes	0 1	—	—	1 7
Neutrophiles staff	10 6	2 7	1 5	4 7
Neutrophiles segmented	7 3	6 1	8 3	6 9
Eosinophiles mature	2 4	2 6	2 9	2 9
Basophiles mature	0 9	0 3	0 3	0 5
Monocytes	7 3	1 5	3 4	2 3
Lymphoblasts	—	0 4	0 1	—
Lymphocytes small young	6 5	5 1	4 0	3 0
Lymphocytes small old	33 9	69 6	70 9	68 5
Lymphocytes large young	4 1	3 0	1 6	1 2
Lymphocytes large old	5 9	2 8	1 5	1 9
Lymphocytes total	50 4	80 9	78 1	74 6
Plasmoblasts	1 3	1 5	0 2	—
Plasmoblasts in mitosis	0 4	0 1	+	—
Plasma cells half mature	1 3	1 5	0 8	0 2
Plasma cells mature	17 6	1 6	1 0	0 6
Plasma cells total	20 2	4 6	2 0	0 8

mitoses It would seem reasonable therefore to expect the greatest intensity of the reaction about the 3rd or 4th day of the illness because on the basis of our investigations of the blood and gland puncture picture in rubella we found that the maturation time of blood plasma cells is from 4 to 5 days (Moeschlin (2)) The fact that the number of neutrophiles is so much lower than in the blood proves that the plasma cells are really derived from the spleen not from admixture of blood with the puncture fluid Further the presence of mitoses and of very young forms also contradicts the idea that they may have been added into the spleen from the blood

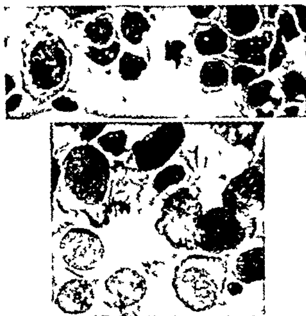


FIG 31 Spleen puncture in infective hepatitis
(a) Numerous lymphatic plasmoblasts and young plasma cells
(b) Mature plasma cells and large lymphatic elements some resembling glandular fever cells

The plasma cell reaction in the spleen diminishes fairly quickly as is shown in the spleen puncture of Cases 2 and 3 *The plasmacytoid reticulum cells are not increased in this stage of the disease they do not appear to increase until the lymphatic reaction is beginning to subside* (Table 14) Also at the same time as the lymphatic plasma cells increase there is a tendency among the lymphocytes for the appearance of forms with wide cytoplasm and young nuclei just as in other types of lymphatic reaction (Fig 31 *b*) These changes are morphologically more striking than is the exact percentage change of the individual cell types Spleen puncture otherwise showed a

typical acute inflammatory reaction viz definite shift to the left of the rather diminished neutrophiles and the presence of a few myelocytes the latter mainly in the late stages in one case they reached 1 7 per cent Erythroblasts were not seen In the later stages, the pulp cells (Cases 2 and 3) as in the majority of inflammatory reactions of the spleen were fairly numerous

The above findings thus show that during and probably even more preceding the presence of numerous large plasma cells in the peripheral blood there are active mitoses of such cells in the spleen from which all types of elements up to the most mature plasma cells pass into the blood It is assumed that the majority of the plasma cells originate in the spleen because there is no similar reaction in the bone marrow (Landolt) It is in conformity with our findings in gland puncture in rubella that the similar cells in epidemic hepatitis are seen to originate from the lymphatic follicles of the spleen Even in epidemic hepatitis a slight enlargement of cervical lymphatic glands may be detectable during the first 5 days although this is always less than in rubella or in infective mononucleosis presumably the difference depends upon involvement of the hepato lienal system in infective hepatitis and the lymphatic glands in rubella

Plasmacytoid reticulum cells are constantly present in the spleen and it is tempting to suppose that young plasma cells arise from them and are really nothing more than specialised functional types We do not share this view because in the early stage of infective hepatitis there is no increase of plasmacytoid reticulum cells in the spleen while Landolt found only normal numbers of these elements in the marrow (as we did also in rubella) He therefore agrees with our contention that the hæmic plasma cells cannot arise from plasmacytoid reticulum cells Equally there is no evidence of transitions from young plasma cells (plasmoblasts) to plasmacytoid reticulum cells

(c) Other Virus Infections Accompanied by Splenomegaly and Lymphatic Reaction

It has already been mentioned that several virus infections may be accompanied by a lymphatic reaction in which irritation cells may be found *i e* elements with wide basophilic cytoplasm or even with monocytoid characters like those of glandular fever cells Doubtless future research will enable the diseases due to lymphotropic viruses to be subdivided but at present this has only been done in the case of mononucleosis (Wising van Berghe) rubella (Steinmaurer) epidemic hepatitis (Siede Luz) and some of the virus pneumonias (Weir Horsfall)

(d) Q Fever (Rickettsia Burneti)

This disease described in Australia by Derrick (1937) runs an acute course and is accompanied by pulmonary infiltrations Gsell

was the first to recognise the disease in Switzerland and it is now our impression that at least 60 per cent of cases of so called virus pneumonia or atypical pneumonia in Switzerland are due to such rickettsiasis

In some cases there is a lymphatic reaction and the spleen is constantly enlarged Spleen puncture in a man of 24 on the 8th day of illness did not reveal any increase of lymphocytes (60 per cent) but many young basophilic types among which mitoses were numerous were seen while there was an associated increase of monocytes (13 per cent) neutrophiles (19 per cent) and plasmacytoid reticulum cells (2 per cent) In the blood the lymphocytes reached their maximum (45 per cent of 7 100 leucocytes) on the 17th day

(e) Lymphocytosis Acuta

Smith (1941) described this disease which usually occurs in childhood Gsell has seen some cases in Switzerland and we have observed two in adults in which the white cells rose to 15–20 000 (all small lymphocytes and no glandular fever cells) but fell to normal again in 2–3 weeks Spleen puncture was not performed because neither lymphatic glands nor the spleen were enlarged but it is reasonable to assume that the number of lymphocytes would be lower than in leukæmic spleens We doubt whether the case to be described in the next section is really an example of lymphocytosis acuta but the possibility cannot be excluded

(f) Non flammatory Lymphatic Reactions (possibly of central origin)

We have seen one case of pituitary tumour in which there was a prolonged and progressive lymphocytosis so great that the condition was originally diagnosed as lymphatic leukæmia Löffler in a personal communication has mentioned an example of extreme lymphocytosis in a similar case

In view of the rarity of such a condition one case in which the enlarged spleen was punctured will be described in more detail especially because 7 years observation have enabled lymphatic leukæmia to be excluded

S H aged 40 had suffered from headaches since a fall in February 1942 there was also diminished libido right sided exophthalmos and left abducens palsy X ray of the skull in March 1942 revealed changes in the sella suggestive of pituitary tumour Blood pictures —

April 17th 1942 leucocytes 4 800 with 59 per cent lymphocytes
June 24th 1942 leucocytes 4 300 with 56 per cent lymphocytes
July 21st 1942 leucocytes 4 200 with 46 per cent lymphocytes
August 19th 1942 leucocytes 18 200 with 79 per cent lymphocytes (14 380 per c mm)

Admitted to hospital on August 25th 1942 with severe headaches and exophthalmos Sella moderately enlarged Spleen just palpable No lymphadenopathy Temperature never above normal E S R 13 mm hæmoglobin 82 per cent red corpuscles 4 1 million

leucocytes 19,800 reticulocytes 1 9 per cent platelets 39 440 neutrophile staffs 5 5 per cent polymorphs 23 5 per cent eosinophiles 3 per cent monocytes 2 5 per cent and lymphocytes 65 5 per cent (12 970 per c mm) The neutrophiles were structurally normal all the lymphocytes small and of old type no basophilic or large forms being found Sternal puncture unsatisfactory but mainly blood was obtained Spleen puncture see below

Later the white cells increased to a maximum of 23 700 with 18 000 lymphocytes After 2 weeks of arsenical treatment by mouth the leucocyte values became normal thus on September 25th 1942 the leucocytes were 4 800 with 34 per cent of lymphocytes As the diagnosis of pituitary neoplasm was uncertain radiotherapy was not tried and the patient was discharged

Re admission February 13th to September 18th 1943 Exophthalmos Tendon reflexes weaker on right bilateral decrease of temporal fields Sella unchanged Lumbar puncture nil abnormal Vision rapidly became worse Leucocytes 9 200 with 27 per cent of lymphocytes Repeated examinations failed to reveal any increase of lymphocytes Radiotherapy to pituitary was followed by gradual improvement of vision and decrease of headaches During an intercurrent attack of pneumonia the leucocytes rose to 16 700 with 5 5 per cent of lymphocytes and this was not followed by a definite post infective lymphocytosis (leucocytes 4 700 with 34 per cent lymphocytes)

No lymphocytosis was found at any time during the subsequent history of the patient but in July 1944 there was transient eosinophilia (27 per cent) In November 1944 the spleen was no longer palpable no enlarged glands could be found and the headaches had ceased while vision was restored to normal The white cells were 6 500 with 20 per cent lymphocytes In January 1945 there was a second attack of pneumonia also not followed by lymphocytosis

In short then this was the case of a man of 40 who developed the typical signs of pituitary tumour and a gradually increasing lymphocytosis (up to 18 000 small mature lymphocytes) with slight splenomegaly The lymphocytosis disappeared after a course of arsenic and has not recurred during a 7 year period of observation—a fact which excludes the possibility that the whole picture could be due to leukæmic infiltration of the pituitary Then again the condition cannot have been a post infective lymphocytosis because its onset was so gradual and its intensity was so great and further no such phenomenon followed the two subsequent attacks of pneumonia Equally a diagnosis of infectious mononucleosis is not tenable on account of the complete predominance of typical small lymphocytes and their gradual increase over a period of months The idea of a very prolonged remission of chronic lymphatic leukæmia was excluded by spleen puncture during the stage of lymphocytosis In that disease (see p 120) the lymphocytes in the spleen are increased to 92–99 per cent often with many immature and polymorphic cells whereas in the present case the splenogram was normal (Table 15) with 85 per cent of lymphocytes rather young but essentially normal cells with very few larger forms

Obviously in view of the transience of the lymphatic reaction it is possible that there was a fortuitous infection with lympho cytosis acuta ' in a person with a pituitary tumour Loffler s observation (mentioned above) and the experimental work of Dougherty and White (1947) seem to us to suggest that a central (pituitary) influence has to be admitted as an explanation It is possible that in association with other endocrine deficiencies there was a decrease of an adrenotropic hormone required by the adrenal cortex The authors just mentioned found that increase *of this hormone caused a fall in the number of lymphocytes* while deficiency leads to increase of lymphocytes varying with the func tional state of the adrenal cortex Only further observations can settle the question

TABLE 15

Hæmog am		Erythroblasts	0 9
Total leucocytes (in 1 000s)	0 7	Myelocytes	0 2
Neutrophiles staff	9	Neutrophiles staff	1 7
Neutrophiles segmented	18½	Neutrophiles segmented	6 0
Eosinophiles	2½	Eosinophiles	2 2
Basophiles	1	Basophiles	0 6
Monocytes	2½	Monocytes	1 6
Lymphocytes	66½	Lymphoblasts	0 4
		Lymphocytes small young	14 2
Splen gram		Lymphocytes small old	66 3
Macrophages	+	Lymphocytes large young	_ 4
Plasmacyt retic cells	0 1	Lymphocytes large old	2 1
Pulp cells	0 7	Lymphocytes Total	85 4
Tissue mast cells	0 1	Plasma cells	0 5

IX CHRONIC INFLAMMATORY SPLENOMEGALIES

The splenic enlargements belonging here may be primarily chronic as in sepsis lenta and Still s disease or may develop out of a preceding acute inflammatory splenomegaly (Fig 32) such as that of malaria Granulomatous conditions although really belonging among the chronic inflammatory states are considered in a separate section

The pathological picture (Lubarsch) is characterised by degenerative changes such as deposition of ferruginous pigment and the occurrence of lipoid cells There are also exsudative phenomena such as hyperæmia with leucocytosis presence of plasma cells and sometimes of hæmo poietic foci with an associated hyperplasia of the reticulo endothelium These changes give the splenogram its characteristic features

Our observations have been on cases of sepsis lenta and of the rheumatic diseases viz Still s and Felty s diseases The literature does not contain detailed descriptions of spleen smears in chronic inflammatory states except in malaria (Nagy 1924 Mele 1925

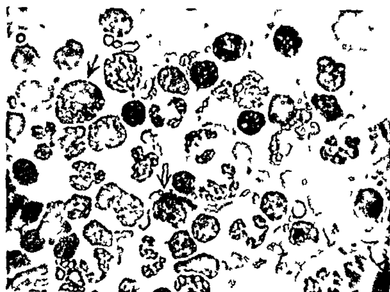


FIG 33 Subacute inflammatory spleen with neutrophiles monocytes occasional myelocytes ↓ erythroblasts ⇑ and lymphopenia

Gosio 1944) and kala azar (Mele Nicolle etc) in which punctures were performed for demonstration of the causative parasites

Weil includes all chronic inflammatory splenograms in the group of macrophage reactions because he regards the presence of large numbers of macrophages as being the characteristic change

Characters of the Chronic Inflammatory Spleen film

The details vary from case to case and with the duration and intensity of the inflammatory process but they have certain features in common —

(1) Increase of plasmacytoid reticulum cells and usually of large lymphatic plasma cells

(2) Increase and shift to the left of the neutrophiles often accompanied by increase of monocytes and lymphopenia

(3) Variable number of erythroblasts and myelocytes

(4) As compared with non inflammatory cases there is excess of pulp cells usually of cell macrophages and sometimes of hæmosiderin macrophages

After a certain amount of practice a general examination of films enables one to recognise a chronic inflammatory spleen (Fig 33 a) and in cases of diagnostic difficulty special attention is to be paid to the finding of the epithelioid cells typical of tuberculosis or the Dorothy Reed cells of Hodgkin s disease The absence of the latter elements in a case of splenomegaly is strong evidence against a diagnosis of Hodgkin s disease because by the time the spleen is enlarged Dorothy Reed cells are usually plentiful in spleen

films On the other hand failure to find epithelioid cells does not exclude tuberculosis because tubercles are irregularly scattered through the organ and may therefore escape puncture

Some neoplasms especially sarcomata accompanied by pyrexia with relative bradycardia and increased E S R *may be accompanied by splenomegaly of chronic inflammatory type probably as a result of protein destruction*

In one case of sarcoma of the retroperitoneal muscles with fever about 100 spleen puncture showed 6 5 per cent plasmacytoid reticulum cells and 23 per cent neutrophiles although at autopsy the distinctly enlarged spleen did not contain any metastases Clinically the diagnosis lay between tuberculosis and Hodgkin s disease but the early onset of cachexia suggested neoplasia

In another case with a chronic inflammatory type of splenogram and high fever caused by lympho sarcoma tumour cells were demonstrable in a second puncture of the spleen

It is worth stressing the value of cultures from spleen punctures especially in those more chronic infections in which repeated blood cultures have been unsuccessful

(a) Sepsis Lenta

In this condition the characters of the chronic inflammatory splenogram are particularly well marked (Table 16) Thus Case 4

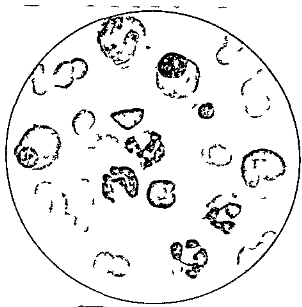


Fig 33 *a*

Chronic inflammatory spleen showing monocytosis plasmacytoid reticulum cell occasional erythroblasts myelo ytes and dim nution of lymphocytes (From the original drawing of Mrs Bollinger Schudel)

in which puncture was performed during a prolonged afebrile period showed 10 5 per cent of plasmacytoid reticulum cells (normal is 0 2–0 8) a figure only once exceeded in our experience in a case of hæmolytic anæmia associated with brucellosis and in one case of Still s disease

In most cases the films contain an excess of pulp cells which are often seen as small syncytia of 2 or 3 cells (Fig 12 c) On account of their irregular distribution the macrophages do not necessarily appear to be increased in the splenogram but in a general examination of films such elements containing cell debris are noticeable In addition, hæmosiderin macrophages may be numerous as may large monocytoid macrophages (Fig 16 i k) sometimes (Case 2) with plentiful large lymphatic reticulum cells In Case 4 we were also able to detect macrophages containing bacteria (Fig 15 c) Neutrophiles vary in number according to the stage of the inflammatory process but always show a shift to the left and sometimes distinct toxic granulation *Monocytes* are considerably increased especially in the more prolonged cases but the percentage cannot be directly compared with that in the blood because lymphocytes are so numerous in the spleen *For this reason any figure above*

TABLE 16

Sepsis Lenta

Case number	*1*	*2*	*3*	*4*
Hæmogram				
Leucocytes (in 1 000s)	1 9	9 7	9 1	11 8
Myelocytes	—	1	—	—
Neutrophiles staff	31½	25½	24½	20½
Neutrophiles segmented	13½	40½	50	39
Eosinophiles	2	—	—	1
Basophiles	—	—	2	—
Monocytes	20	9	5	18½
Lymphocytes	33	24	18½	21
Splenogram				
Macrophages	0 8	0 3	0 1	0 1
Plasmacyt retic cells	*1 6*	*3 0*	*1 4*	*9 3*
Pulp cells	6 8	1 5	0 3	2 5
Tissue mast cells	+	—	—	—
Fat cells	+	+	—	—
Erythroblasts	—	+	—	+
Myelocytes	—	1 4	—	0 1
Neutrophiles staff	5 8	7 9	5 0	8 8
Neutrophiles segmented	3 9	6 2	11 9	19 5
Eosinophiles mature	0 8	—	0 3	1 8
Basophiles mature	0 6	0 1	0 4	0 1
Monocytes	1 1	0 7	3 2	7 1
Lymphoblasts and large lymphatic reticulum cells	0 2	1 3	0 8	—
Lymphocytes small	72 2	63 3	63 2	46 7
Lymphocytes large	4 8	11·9	12 4	2 9
Lymphocytes total	77 0	76 5	76 4	49 6
Plasma cells	0 9	2 4	1 0	0 7

3 *per cent is to be regarded as an excess* As a rule the lymphocytes tend to be rather younger and larger than normal even a few baso philic ones being present *Myelocytes* and *erythroblasts* although less numerous than in acute cases are constantly found

In one case we found many *tissue mast cells* (Fig 16 *g h*) and in two cases (Case 1 and 2) lipophages were numerous

It is to be noted that films from chronic inflammatory spleno megaly (Fig 33 *a*) differ from normal in general appearance more than is shown by the numerical changes in the splenogram

These findings in spleen films from Libman Sacks disease are the same as in sepsis lenta

(b) Still Chauffard Felty Disease

This disease which belongs to the rheumatic group (Spuhler Zimmer) runs a chronic course accompanied by polyarthritis and splenomegaly which may become considerable Sooner or later anæmia leucopenia and thrombocytopenia develop as a resu t of splenopathic inhibition of myeloid function while gradually progressive hæmochromatosis is also characteristic

Splenectomy may cause improvement especially of the blood picture (Hatch Büchler Zimmer Hirschboeck and our own observations)

In our own opinion there are no grounds for separating Still s disease from Felty s syndrome the enlargement of lymphatic glands that occurs in the former condition but not in the latter depends mainly on the age of the patients Some relationship certainly exists between these diseases and Libman Sacks disease and also cyclical agranulocytosis In some typical cases of the Still

TABLE 17

Morbus Still Chauffard Felty

Case	1 44 ♂	2 35 ♀	Case	1 44 ♂	2 35 ♀
Hæmogram			Pulp cells	3 1	1 4
Total leucocytes	2 400		Erythroblasts	0 2	+
Neutrophiles staff	45		Myelocytes	0 5	0 6
Neutrophile segmented	29		Neutrophiles total	21 7	16 6
Eosinophiles	3		Neutrophiles staff	12 0	13 0
Basophiles	1½		Neutrophiles segmented	9 7	3 6
Monocytes	6		Eosinophiles	0 1	0 4
Lymphocytes	15½		Basophiles	0·5	0·4
Plasma cells	—		Monocytes	2 0	2 0
Myelogram			Lymphoblasts	—	—
Plasmacyt retic cells			Lymphocytes small young	5 1	2 4
(per 100 leucocytes)	14 4		Lymphocytes small old	57 6	58 6
Lymphoid retic cells	38 8		Lymphocytes large young	0 7	0 4
Splenogram			Lymphocytes large old	1 5	2 8
Macrophages	0 6	1 2	Lymphocytes Total	59 9	64 2
Plasmacyt retic cells	10 3	12 4	Plasma cells	1 1	0 8

Chauffard Felty syndrome definite agranulocytic episodes occur periodically all 3 weeks (see next chapter)

We are in a position to report on 4 cases (1 man and 3 women) in all of which the spleen films were of the chronic inflammatory type with increase of plasmacytoid reticulum cells (up to 12 4 per cent in Case 2) and of monocytes In one patient (Table 18 *a*) eosinophils and lymphocytes were more numerous than in our other cases although many authors regard lymphatic hyperplasia sometimes with eosinophilia as characteristic of the disease

Case 1 presents a number of important features and will there fore be recorded in some detail

P J male aged 44

1935-36 in *hospital for a long period suffering from rheumatic* polyarthritis 1936 polyserositis (? rheumatic)—guinea pig inoculation negative 1937 further attack of pains in joints enlargement of glands spleen just palpable distinct leucopenia

Diagnosis Still Chauffard Felty disease—gradual improvement but persistent pains and raised E S R General condition poor even slight scratches fester—in November 1940 such an infection of a finger led to lymphangitis fistula in the axilla until patient admitted to hospital in 1943

A thin man with atrophic skin no glandular enlargement and much pain in joints with slight swelling Slight pyrexia Spleen palpable (12 cm) and firm Discharging sinus in right axilla—all examinations for T B negative

Blood red corpuscles 3 1 hæmoglobin 72 per cent reticulocytes 1 4 per cent leucocytes 2 400 lymphocytopenia of 15 5 per cent B S R persistently high 68 mm Takata + + Weltmann 0 35 serum protein 7 5 (upper limit of normal)

Sternal puncture great increase of plasmacytoid reticulum cells viz 14 4 per cent lymphoid reticulum cells also greatly increased—98 8 per 100 white cells Slight shift to left of neutrophiles

In hospital for 8 months but treatment had little effect In 1945 he was admitted to another hospital in much the same condition no bone changes

Spleen puncture revealed great increase of plasmacytoid reticulum cells (10 3 per cent) as shown in Table 17 (Case 1) In addition to the typical appearances of a chronic inflammatory splenomegaly there were slight lymphocytopenia moderate monocytosis increase of neutrophiles and distinct increase of macrophages In distinction from the sternal marrow there was no increase of other reticulum cells which in the marrow was presumably attributable to a reactive proliferation resulting from chronic inflammation (see later in the discussion of reticulosis)

The remarkable increase of plasmacytoid reticulum cells in the spleen and marrow was associated with a much increased B S R (68-72 mm) and a persistently positive Takata reaction Even so myeloma could be excluded because of the clinical course and the normal characters of the plasmacytoid reticulum cells

Our view is that the blood changes are related to increase of certain globulin fractions which are doubtless formed at least to a considerable extent by the plasmacytoid reticulum cells of the marrow and spleen Perhaps the small lymphoid reticulum cells also play a part in the increased formation of globulins although these elements do not seem to increase primarily but only reactively Waldenstrom s (1948) observations on diffuse proliferation of reticulum cells in the marrow associated with the presence of globulin of high molecular weight in the blood are probably sig nificant in this connection We have to ask ourselves if this case does perhaps belong to the above group as well but unfortunately it cannot be decided yet

(c) **Cyclical Agranulocytosis in the Felty Syndrome**

This is a remarkable condition in which at fairly regular intervals of 3 weeks there are febrile attacks accompanied by neutropenia and such episodes may continue for years Two forms of the malady

TABLE 18a

Cyclical Agranulocytosis (Male aged 20)

Date	5/42	1 /44	1/45	Date	5/4	12/44	1/45
	Puncture		Film		Puncture		Film
Hæmogram				Erythroblasts	—	—	—
Total leucocytes				Myelocytes	—	—	—
(in 1 000s)	5 1	4 8	3 4	Neutrophiles staff	2 9	0 1	0 3
Neutrophiles staff	12½	1	3	Neutrophiles segmented	3 2	0 1	0 6
Neutrophiles segmtd	9	1	7	Eosinophiles	4 3	4 1	2 2
Eosinophiles	10	18½	11	Basophiles	0 3	0 4	0 7
Basophiles	½	—	2	Monocytes	8 3	3 9	6 4
Monocytes	27	23	24	Lymphoblasts	0 3	—	0 1
Lymphocytes	41	56½	53	Lymphocytes small young	7 6	4 8	10 9
Plasma cells	—	—	—	Lymphocytes small old	69 6	81 0	71 4
				Lymphocytes large young	1 9	1 5	1 6
Splenogram				Lymphocytes large old	1 7	2 9	4 6
Macrophages	+	0 2	0 1	Lymphocytes Total	80 6	90 [illegible]	88 6
Plasmacyt retic cells	0 2	0 3	0 4	Plasma cells	0 2	0 5	0 5
Pulp cells	—	0 2	0 2	Eosinophiles per 100 gran	22½	49	0½

have to be distinguished viz one with periodic oral ulcers but no polyarthritis and an arthritic type

We have records of a man now aged 26 in whom these pheno mena have been observed for 6 years It was a typical case of the Still Chauffard Felty syndrome with a large spleen chronic poly arthritis and gradually progressive hæmochromatosis associated with rheumatic endocarditis of the mitral valve Every 3 weeks there was an attack of neutropenia with increase of monocytes and aggravation of the arthritis During such phases sternal punc ture showed distinct immaturity with disappearance of the more mature granulocytes

This case has been published in detail by Loffler and Maier and a very similar one has since been recorded by Muller and Meyrieux (1948) Then as already mentioned there are cases with no rheumatic manifestations but with oral ulcers in the agranulocytic stage (Imerslund 1942 Vahlquist 1946 Reimann 1948 Barling 1948) In the latter type of case the spleen does not seem to be enlarged It seems probable that the cases with polyarthritis are nothing more than cyclical agranulocytoses complicated by rheumatism

Spleen punctures which were performed during apyrexial freedom from symptoms revealed distinct increase of eosinophiles on both occasions viz 4 3 and 4 1 per cent (normal is 0 2–1 5 per cent) If the number of eosinophiles is calculated relative to the total granulocytes it will be found that on the first occasion there were 40 per cent and on the second 21 per cent Such eosinophilia could also be confirmed in 1945 (2 2 per cent *i e* 18 eosinophiles per 100 granulocytes) and by histological examination of the spleen Apart from distinct increase of monocytes and lymphocytes puncture fails to reveal other changes and there was certainly no increase of plasmacytoid reticulum cells

The splenic and hæmic eosinophilia in the absence of any evidence of parasite infestation strongly suggested an allergic basis for the disease

(d) Malaria

Owing to the rarity of malaria in Switzerland we have records of only two punctures Case 1 was a severe chronic tertian infection with great splenomegaly in which no treatment had been given Case 2 was that of a man who had been infected with tertian malaria about a year earlier but who in spite of freedom from relapses still had a large hard spleen

In both cases we found macrophages containing melanin and plasmodia as described by Melé (1925) and Gosio (1944) As long ago as 1924 Nagy pointed out that the parasites might easily be found by spleen puncture even in cases in which repeated examination of the blood had failed to reveal them And as Gosio stresses the finding of the typical malaria pigment is of special importance in diagnosis This is dead black unlike the greenish yellow of hæmosiderin which is of course increased in amount in all chronic hæmolytic conditions Fig 33 *b c* shows such malaria macrophages (from Case 2) filled with large aggregations of black pigment (This should be compared with the larger pigmented cells of the malignant melanoma (Fig 53 *f g*) which have a much broader cytoplasm) Malarial pigment may also be found in larger and smaller cells as well as ingested by granulocytes (Gosio) while it and hæmosiderin may also be seen lying free in films *The presence of melanin*

in spleen puncture can confirm a diagnosis of malarial splenomegaly even when no parasites can be found

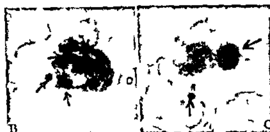


Fig 33 *b c* *Malaria melanin*
Macrophages contai in melanin Dark black rounded masses of pigment are characteristic and can easily be distinguished from the yellowish green or brownish yellow tint of hæmosiderin (spleen puncture)

Demonstration of the Parasites Some of the parasites lie inside red corpuscles while others lie in a rosette arrangement round the nuclei of macrophages Gosio stresses the fact that no parasite may be discoverable in the spleen between attacks although he did find quartan ones even after months of freedom from recurrence During attacks he observed macrophages that had ingested red corpuscles which themselves contained parasites

Spleen punctures regularly show an increase of plasmacytoid reticulum cells some erythroblasts and myelocytes with excess of granulocytes and monocytes a typical chronic inflammatory splenogram

TABLE 18b
Malaria

Case	Macrophages	Melanin *macrophages*	Plasmacyt retic cells	Pulp cells	Metamyelocytes	Neutrophilic Staff	Neutrophilic Segmented	Eosinophiles	Monocytes	*Lymphocytes* Young	*Lymphocytes* Old
1	–	+	3 0			32 0	30 4			0 2	29 8
55 ♂	3 0			1 6	1 8	62 4		–	1 2	30 0	
2	3 5	+	++			14 0	16 0			2 0	59 0
33 ♂	3 5			3 0	—	30 0		0 5	2 0	61 0	

(e) Kala azar (Leishmaniasis interna)

Until recently most spleen punctures have been performed for the demonstration of *Leishmania donovani* After a prolonged period of septic pyrexia leukopenia and anæmia and sometimes enlargement of lymphatic glands this disease which is indigenous to the Mediterranean countries South America and Asia leads to great splenomegaly (Nicolle Benhamou etc)

The parasites both lying free and in macrophages are numerous in spleen films Weil describes them as follows —



Fig 33 d *Kala-azar*

Typical leishmanias can be seen in a group of macrophages in a typical chronic inflammatory spleen puncture A small punctate centrosome can also be clearly seen ( ) in every leishmania

In spleen films the causative organisms (2 4μ) are oval rounded or pyriform These organisms which are always numerous are mainly intracellular although some are seen scattered in small groups which somewhat resemble masses of platelets Careful observation however reveals distinct differences Thus they are each surrounded by a delicate transparent membrane which encloses pale blue cytoplasm which contains 2 small differentiated structures The larger rounder one which lies eccentrically is the violet nucleus while the smaller basophilic one usually staff shaped is the centrosome

We owe the films from which the following splenograms were made to the kindness of Dr Farreras of Barcelona

The changes are those characteristic of a chronic inflammatory spleen although lymphocytes are rather more numerous than usual over shadowing the monocytes and neutrophiles

In Case 1 there is great increase of plasmacytoid reticulum cells which seems to be characteristic of the disease both in the spleen and the sternal marrow (Leitner) This is accompanied by an

TABLE 18c

Kala a ar

Case	Macrophages	Leishmann—microphages	Plasmacyt retic cells	Pulp cells	Erythroblasts: Basophilic	Polychromatic	Oxyphil c	Myelocytes: Half mature	Mature	Meta	Neutrophiles: Staff	Segmented	Eosinophiles	B sophiles	Monocytes	Lymphocytes: Young	Old	Lymphatic plasma cells
1	0 5	1 3	6 8		—	0 5	1 ?	—	0 3	?0	4 7	2 0				3 7	75 6	
	8 6			—	1 7			3			6 7		—	—	0 8	79 3		0 5
2	0 8	+	1 0		0 2	0 4	1 2	0 6	—	3 2	10 0	1 0				2 4	75 6	
	1 8			0 6	1 8			3 8			11 0		0 2	0 2	-6	78 0		—

increased B S R The macrophages were mainly seen in groups in the films

Gatto also saw increase of plasmacytoid reticulum cells and a slight extramedullary myelopoiesis (28 cases)

The occurrence of splenopathic inhibition of the marrow in chronic cases with great splenomegaly is an occurrence to be borne in mind (Cartwright 1948)

(f) Histoplasmosis

We have not had the opportunity of examining a case but presume that the parasites would be found inside reticulum cells in the spleen The organisms show 2 or 3 large rounded structures resembling a saucer 1 to 10 being found in each affected macrophage (see Wintrobe Plate XIII) but as far as we are aware they have up to the present time only been demonstrated histologically

X GRANULOMATOUS CONDITIONS

Diseases in which the causative organisms evoke the formation of a specific type of granulation tissue belong to this group We shall however only discuss tuberculosi sarcoidosis syphilis and undulant fever leaving Hodgkin s disease the nature of which infective or neoplastic to a separate section

(a) Tuberculosis

A definite diagnosis of tuberculous infection can be made by spleen puncture only if elements characteristic of the disease are

found in epithelioid or Langhans giant cells or if the bacteria can be demonstrated by staining or culture Our own observations on gland puncture as well as those of Stahel and Tischendorf indicate that the characteristic changes do not develop in less than 2 or 3 weeks after infection

We have records of 5 cases in which the specific elements were found in spleen films Nassau (1926) recorded a case of sepsis with splenomegaly in a child in which a diagnosis of miliary tuberculosis was made by finding tubercle bacilli in films from spleen puncture Weil (1936) aspirated caseous material in which no tubercle bacilli could be found although it was capable of infecting a guinea pig

Epithelioid and giant cells have been found in gland puncture by Klima and Fleischhacker (1937) Tischendorf (1939) Stahel (1939) Leitner (1940) and Forteza (1947) but there are no previous records of their being found in spleen punctures

Structure of Epithelioid and Langhans' Giant cells

Epithelioid cells (20–35 μ) are usually seen as elongated oval or slightly pointed elements often lying in small groups The oval pale violet reddish or deep violet nucleus (14–20 μ) consists of a characteristic arrangement of narrow rods of chromatin with intervening spaces such that one has the impression of looking through a fine network (Fig 34 *a*) In the younger forms a small pale blue or violet nucleolus may be seen but this is not visible in the older cells which may indeed show some degree of pyknosis leading to a coarser arrangement of the chromatin (Figs 34 *b* and *c*)

The pale blue or greyish cytoplasmic ring is relatively wide in young cells and becomes paler and more violet in older ones (Fig 35) which may contain vacuoles and fine violet granules The edge of the cell body is rather indistinct so that when these elements lie in groups it may not be possible to separate them

The multi nucleated Langhans giant cells present the same kind of nuclear structure as that of epithelioid cells but more often with some pyknosis Stahel believed that these elements as seen in gland puncture arise from fusion of the epithelioid cells In our opinion these cells probably arise from toxic inhibition of mitosis the nuclei undergoing division without division of the cytoplasm (polyploid cells) as in the case of Sternberg cells of Hodgkin s disease and multinucleated tumour cells (see also p 43) Thus it is possible to find every transition between cells with 2 or 3 nuclei and enormous elements (70–90 μ) with the characteristic palisade arrangement of their numerous nuclei In the cell shown in Fig 36 it is possible to distinguish 41 nuclei but the typical arrangement found in sections may be disturbed in the making of films

Both epithelioid and Langhans cells can be distinguished from Dorothy Reed cells because the nuclei of the latter are larger and rounder while the chromatin has a more granular arrangement



FIG 34 Tuberculous epithelioid cells from tuberculosis of the spleen

(a) Typical young type
(b) Older form
(c) Disintegrating cell
(d) Fusion of e eral epithelioid cells to form a multi nucleated elem nt
(e) Langhans giant-cell from chronic tuberculosis of the spleen

with one or more distinct dark blue violet nucleoli while the cytoplasm is blue as compared with the pale greyish violet of that of epithelioid cells (Plate II Fig 37)

We can report on the *splenograms* of 7 cases (Table 19) which can be classified as follows —

(1) Acute hæmatogenous tuberculosis

(2) Miliary tuberculosis

(3) Chronic tuberculosis of the spleen

(1) *Acute Hæmatogenous Tuberculosis* In one severe case with prolonged fever leucocytosis and splenomegaly in which tubercle bacilli were grown from a pleural effusion the splenogram was characteristic of an acute inflammatory reaction with neutrophilia *shift to the left and lymphocytopenia (see pp 80 and 81, Table 12,* Case 1) There is in fact no difference between such splenogram and that of any other acute inflammatory one Stahel and Tischendorf found a similar state of affairs in the early stage of glandular tuberculosis viz a predominantly leucocytic response

TABLE 19

Cases of Tuberculosis

Case	1	2	3	4	5
Age Sex	30 +	38 ♂	61 ♀	18 ♂	28 ♂
Hæmogram					
Total leucocytes	3 400	—	12 600	—	—
Neutrophiles staff	75½	—	18½	—	—
Neutrophiles segmented		—	68½	—	—
Eosinophiles	4½	—	—	—	—
Basophiles	—	—	—	—	—
Monocytes	8½	—	10½	—	—
Lymphocytes	11½	—	2	—	—
Plasma cells	—	—	—	—	—
Splenogram					
Macrophages	0 4	0 2	0 1	—	0 4
Plasmacyt retic cells	1 4	1 0	3 1	0 1	0 2
Pulp cells	1 5	—	1 0	1 1	[illegible] 2
Erythroblasts	0 2	—	—	—	—
Myelocytes	0 5	—	0 5	—	0 2
Neutrophiles staff	17 7	3 9	2 6	14 1	5 6
Neutrophiles segmented	9 4	11 8	11 9	14 6	5 4
Eosinophiles	2 5	1 2	—	1 5	1 6
Basophiles	0 1	0 3	0 1	0 4	0 6
Monocytes	20 3	3 6	3 4	7 4	2 2
Lymphoblasts	—	—	—	—	—
Lymphocytes small young	0 7	0 3	2 8	3 1	2 4
Lymphocytes small old	41 7	75 8	71 3	53 8	75 0
Lymphocytes large young	0 2	0 6	0 6	1 4	0 4
Lymphocytes large old	0 7	1 [illegible]	2 4	1 5	[illegible] 2
Lymphocytes Total	43 3	77 9	77 1	59 8	80 0
Plasma cells	0 3	0 1	0 2	0 6	0 [illegible]
Epithelioid cells	2 3	+	+	0 4	1 4
Langhans giant-cells	0 1	—	—	—	—

PLATE II PATHOLOGICAL FORMS OF RETICULO-ENDOTHELIAL CELLS IN SPLEEN FILMS (From paintings by Mrs Bollinger Schudel)



FIG 35	Epithelioid cells of tuberculosis
FIG 36	Langhans giant-cell
FIG 37	Young Dorothy Reed (Sternberg) cell with typical blue nucleoli (Hodgkin s disease)
FIGS 38 39	M lti nucleated giant-cell Dorothy Reed cells
FIG 40	Pathological reti ulum cells (neoplastic) from a case of primary *malignant reticulosis (reticulo-endotheliosis)*

[F l g pag 106

with one or more distinct dark blue violet nucleoli while the cytoplasm is blue as compared with the pale greyish violet of that of epithelioid cells (Plate II Fig 37)

We can report on the *splenograms* of 7 cases (Table 19) which can be classified as follows —

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TABLE 19

Cases of Tuberculosis

Case	1	2	3	4	5
Age Sex	30 ♀	38 ♂	61 ♀	18 ♂	?8 ♂
Hæmogram					
Total leucocytes	3 400	—	12 600	—	—
Neutrophiles staff	75½	—	18½	—	—
Neutrophiles segmented		—	68½	—	—
Eosinophiles	4½	—	—	—	—
Basophiles	—	—	—	—	—
Monocytes	8½	—	10½	—	—
Lymphocytes	11½	—	2	—	—
Plasma cells	—	—	—	—	—
Splenogram					
Macrophages	0 4	0 2	0 1	—	0 4
Plasmacyt retic cells	1 4	1 0	3 1	0 1	0 ?
Pulp cells	1 5	—	1 0	1 1	– ?
Erythroblasts	0 2	—	—	—	—
Myelocytes	0 5	—	0 5	—	0 2
Neutrophiles staff	17 7	3 9	2 6	14 1	5 6
Neutrophiles segmented	9 4	11 8	11 9	14 6	5 4
Eosinophiles	2 5	1 2	—	1 5	1 6
Basophiles	0 1	0 3	0 1	0 4	0 6
Monocytes	20 3	3 6	3 4	7 4	2 ?
Lymphoblasts	—	—	—	—	—
Lymphocytes small young	0 7	0 3	?8	3 1	2 4
Lymphocytes small old	41 7	75 8	71 3	53 8	75 0
Lymphocytes large young	0 2	0 6	0 6	1 4	0 4
Lymphocytes large old	0 7	1 2	2 4	1 5	2 2
Lymphocytes Total	43 3	77 9	77 1	59 8	80 0
Plasma cells	0 3	0 1	0 2	0 6	0 2
Epithelioid cells	2 3	+	+	0 4	1 4
Langhans giant-cells	0 1	—	—	—	—

(2) *Miliary Tuberculosis* The splenogram of Case 3 was from a woman of 61 with chronic pulmonary tuberculosis who suddenly developed pyrexia and a palpable enlargement of the spleen A suspicion of miliary tuberculosis was naturally aroused but the diagnosis was not definitely confirmed by radiography

Spleen films were typical of a chronic inflammatory condition viz increase of plasmacytoid reticulum cells (3 1 per cent) pulp cells myelocytes (0 5 per cent) and slight monocytosis without increase of neutrophiles At one place in the film 2 typical epithelioid cells were seen (Fig. 34 *a*) The further course of the illness and the autopsy at which recent tubercles were found in the spleen confirmed the diagnosis

(3) *Chronic Tuberculosis of the Spleen* Some details of Case 1 which was of exceptional interest will be given

G K aged 30 saleswoman Not well since pleurisy with effusion at age of 30 Irregular fever up to 101 F heaviness in upper abdomen and enlargement of the spleen

In hospital the temperature varied up to 102 F sweating spleen greatly enlarged reaching iliac crest Liver enlarged (15 cm) Liver function tests were within normal limits Mantoux 1 100 000 negative 1 10 000 positive W R negative Brucella test negative B S R 43 mm

Blood Hæmoglobin 76 per cent reds 3 5 million reticulocytes 1 6 per cent constant leukopenia 3 400 neutrophils 77 per cent eosinophiles 3 5 per cent (sometimes up to 8 per cent) monocytes 6 per cent lymphocytes 13 5 per cent platelets 76 000

Sternal Puncture 15 6 nucleated red cells per 100 white cells slight immaturity of granulopoiesis and some increase of megakaryocytes

Hodgkin s disease was suspected but radiotherapy neither improved the general condition nor reduced the size of the spleen

Spleen Puncture Typical epithelioid cells found and tuberculous infection was also confirmed in an excised cervical lymphatic gland

Splenectomy was performed in the Surgical Clinic (Prof Clairmont) 28 cm long and 1 740 g in weight *Histologically* (Pathological Institute Zurich Prof von Meyenburg) the serous coat was smooth but greyish nodules could be seen through it On section there were many submiliary greyish nodules which in some areas were in groups Microscopically these were composed of epithelioid and Langhans giant cell only remnants of pulp with dilated sinuses lay between the nodules The *pulp cords were infiltrated with plasma cells and to a less extent with* eosinophiles and neutrophiles No follicles could be recognised

Thus this is a case of isolated nodular tuberculosis of the extremely enlarged spleen (28 cm and 1 740 g) with a long period of intermittent fever Spleen puncture revealed the pre ence of epithelioid cells and a splenogram characteristic of a chronic inflam matory reaction with an unusual degree of monocytosis (20 per cent instead of the normal maximum of 2 5 per cent) Another case has already been discussed in the section on thrombocytopenic

culosis splenectomy with prophylactic streptomycin treatment is the therapeutic of choice

b Sarcoidosis (Boeck)

In about one third of the cases of pulmonary sarcoidosis the spleen is also involved (Loffler and Dressler Loffler and Jaccard) but the spleen may be the only affected organ (Secretan Gebsattel Askanazy Friedmann)

We have no experience of spleen puncture in a proven case of

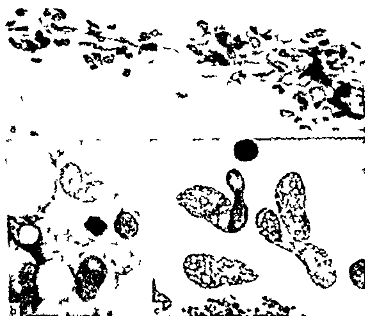


Fig 41 a b c Epithelioid cells

(a and b) Sarcoidosis (Boeck) general and detail views Delicate network of chromatin and distinct nuclei and arrangement of the cells in draught of fishe (gland puncture)

(c) Morbus Bang Note the large size of the cells and the relatively large masses of chromatin (spleen puncture)

sarcoidosis and are not aware of any reports in the relevant literature In one doubtful case (Case 5 Table 19) with hilar lymphomata spleen puncture revealed many young epithelioid cells but as the Mantoux test was positive (1 100 000) a definite diagnosis could not be made

Histologically sarcoid granulomata in the spleen and other organs closely resemble tuberculosis (Loffler Dressler) but generally without any sign of caseation

In one patient with hilar lymphomata we found the characteristic epithelioid cells in groups and garlands by puncture of

splenomegaly (p 77) Case 4 which we owe to the kindness of Professor Rohr showed numerous epithelioid cells in its chronic inflammatory type of splenogram

Summary In *recent miliary spread* spleen films are of acute or subacute inflammatory type viz increase of neutrophiles with leftward shift some myelocytes and perhaps monocytosis and lymphocytopenia At this stage diagnosis has to depend upon the finding of acid fast bacilli because the characteristic granulation tissue has not yet been formed

In *more prolonged cases* of hæmic spread spleen puncture shows ordinary chronic inflammatory changes with increase of plasmacytoid reticulum cells and perhaps, neutrophilia with shift to the left monocytosis and lymphocytopenia Of course if the needle penetrates the specific granulation tissue there will be many epithelioid cells and sometimes giant cells Such appearances with striking monocytosis were found in Case 1 already described

Thrombocytopenic purpura probably of mixed toxi infective and hormonal origin (inhibition of maturation of megakaryocytes by hypersplenism) is by no means uncommon in association with tuberculosis of the spleen (see Case 2 Table 11 and also reports of Kellert Zorini Alessandri Weiner Reggiani and Lapp)

Diagnostic Value Spleen puncture is in our opinion of special value in cases of tuberculosis confined to the spleen because by examination of several films (and repeated puncture if necessary) it will usually be possible to demonstrate epithelioid cells But even in miliary cases which often cause diagnostic difficulties spleen puncture may be useful always remembering to stain at least one film for acid fast bacilli which in Nassau s case made a difficult diagnosis clear It is of course also valuable to use some of the puncture fluid for culture and animal inoculation

In cases of persistent pyrexia with splenomegaly in which malaria sepsis lenta chronic cholangitis and undulant fever can be excluded failure to find signs of specific granulation tissue in an otherwise typical chronic inflammatory splenogram is in favour of tuberculosis rather than of Hodgkin s disease which is usually so diffuse as to be demonstrable in repeated puncture If cachexia be pronounced with fever increased B S R and perhaps a chronic inflammatory splenogram the possibility of sarcoma will occur to the mind (see p 95)

Weil and Perles (1938) have recorded cases of splenic tuberculosis with intense erythroblastic reaction in the spleen and they have included these in their group of crypto erythroblastoses It is possible that they were really dealing with the fairly common condition of tuberculosis complicating chronic myeloid leukæmia (see erythroleukæmias p 172)

Therapeutic In all cases of confirmed chronic spleen tuber



FIG 41 *d e* *Syphilitic epithelioid cells*

Puncture of an inguinal gland in a case of primary syphilis during improvement I owe the preparation to the kind co-operation of Dr Ludin (*d*) Young types with delicate transparent chromatin network (*e*) Multi nucleated giant cells (Those shown are a rather older type with close chromatin) The nuclei are somewhat larger and the cytoplasm wider than that in the epithelioid cells of tuberculosis

bluish grey without any granules In places it was possible to see these cells in process of forming multi nucleated giant cell (Fig 41 *e*) Forteza saw only a hyperplastic reaction in an inguinal gland of a primary sore and chronic inflammation with many plasmacytes in a necrotic gland (Stuyt Strunge Pavlovsky) Although no spleen punctures have been recorded it seems certain that a picture similar to that in lymphatic glands *i e* with epithelioid cells would be found

(d) Morbus Bang

We have records of 7 spleen punctures and 1 smear In 5 cases (4 punctures and 1 smear) we found large reticulo endothelial cells (Fig 41 *c*) which were almost certainly identical with the fusiform

a cubital lymphatic gland and the diagnosis of sarcoidosis was also confirmed by histology (Fig 47 *a b*) Stahel (1939) and Leitner (1940) had also recorded diagnosis of this disease by gland puncture It seems reasonable to suppose that the spleen would show similar appearances

The epithelioid cells of sarcoidosis are indistinguishable from the younger ones found in tuberculosis but only well preserved ones are found there is never any sign of cellular senility such as pyknosis This is presumably, attributable to the absence of caseation in sarcoidosis There are, however differences of distribution in tuberculosis the epithelioid cells found in gland puncture tend to be arranged in small groups whereas in sarcoidosis their arrangement is in distinct groups or draught of fishes agglomerations If therefore in the absence of positive tuberculin tests this distribution were seen in spleen puncture one would be prepared to make a definite diagnosis of sarcoidosis Some of the puncture material should always be used for sections in order to demonstrate the characteristic nodules Van Beck found such granulomata in liver puncture while Stahel and Gormsen were able to see them in sections of sternal puncture fluid

(c) *Syphilis*

We have no personal observations but splenomegaly sometimes associated with syphilitic cirrhosis may occur in both the congenital and the acquired disease (Naegeli Curschmann, etc) Inhibition of marrow function may ensue but can often be cured by anti luetic treatment although splenectomy may be required (case of Wintrobe)

The appearances of the specific epithelioid and giant cells have not yet been recorded in the literature but Ludin did describe the characters of gland puncture in a paper read at the Swiss Hæmatological Society in 1948 (inguinal gland of a man aged 26 with a healing primary sore roseolar rash and positive W R) By his kindness we have been able to examine and photograph the films (Fig 41 *d e*) Ludin found epithelioid and giant cells but in addition there were signs of chronic inflammation with many lymphocytes showing every stage of maturation from lymphoblasts to mature cells together with many reticular elements of the type of sinus endothelium some of which showed evidence of phagocytic activity In addition there were many plasma cells of all ages from plasmoblasts to mature forms

Syphilitic epithelioid cells (Fig 41 *d*) closely resemble the younger ones found in tuberculosis their nuclei are rather thicker and larger with very fine chromatin in the younger cells Unlike Hodgkin cells there is only one small bluish red nucleolus The cytoplasm is broader than in the epithelioid cells of tuberculosis and is pale



Fig 41 f Blastomycosis

Pa acocc d o d s brasiliensis Spl n l re de Almei la Disease Large giant cells which arise by fusion of epithelioid cells are here seen containing the typical fungi which are seen as the refractive spheres with a typical double outline

It would be reasonable to believe that in other fungus diseases of the spleen *e g* coccidioidomycosis the fungi could be demon strated in the spleen puncture To our knowledge no such reference has yet been made

C HODGKIN S DISEASE

We are inserting this section between those on inflammatory and neoplastic conditions because Hodgkin s disease cannot defi nitely be assigned to either group although on the whole American writers incline to the neoplastic theory (for literature see Ratkoczy)

The spleen is often clinically affected (Naegeli) while patho logically it is frequently enlarged and widely replaced by the charac teristic granulation tissue (Lubarsch) Indeed cases in which the disease is confined to the hepatolienal system for a long time are not uncommon and of course present much diagnostic difficulty (Pedro Pons Farreras Valenti)

Introzzi was the first to use spleen puncture as a diagnostic method while Storti (1935) Weil (1936) Heilmeyer (1942) and Farreras Valenti (1947) have also recorded cases in which the finding of the characteristic Dorothy Reed cells led to a diagnosis

The first descriptions of the character of these cells as seen in films are due to Guthrie (1921) and Introzzi (1932) and their discovery in films from gland puncture has often been recorded *e g* Pavlovsky (1934) Weil (1936) Fleischhacker and Klima (1937) Tischendorf (1939) Storti (1939) Stahel (1939) Forteza (1947) Ludin (1948) etc

epithelioid cells which were first described in sections by Loffler and von Albertini We have never seen similar cells in any other condition Further the films showed the usual signs of a chronic inflammatory reaction with normal and slightly increased numbers of lymphocytes (75–80 *per cent*)

Structure These cells are striking on account of their size greyish blue cytoplasm indistinct outline and *when seen in groups absence of* intercellular connections The nucleus (17–28 μ) is longish oval or monocytoid with some lobulation The chromatin is reddish violet and *has a reticular arrangement with varying sizes of mesh (see Fig 41 c)* Occasionally a small nucleolus can be seen

We have already discussed the spleen films of a case complicated by hæmolytic anæmia (p 72) in which in addition to cells of this type there were many erythroblasts and a few myelocytes

As we pointed out in our earlier work (Loffler and Moeschlin) it is not uncommon to find a Bang infection manifesting itself by *splenomegaly without any signs of general disease It is therefore* recommended that agglutination tests should be done in every case of splenomegaly of uncertain origin whether accompanied by general symptoms and lymphocytosis or not

Our findings have been confirmed by Sundberg and Gormsen who found the characteristic cells in the sternal marrow

Diagnosis Spleen puncture is not of value as a routine procedure but it can be so in chronic cases (see above) For example Dr Buchler (Tiefenauspital Bern) kindly sent us films from a boy of 17 who had suffered before from a definite abortus infection and who now had anæmia (hæmoglobin 60 per cent) and only a weakly positive *agglutination therefore diagnosis was uncertain and other* diseases had to be *emphasised* The typical Bang cells were found in the films and treatment with collargol (Loffler) resulted in disappearance of all the signs

(e) Mycoses of the Spleen (*Blastomycosis brasiliensis*)

Mycoses of the spleen are extremely rare diseases in Europe *The South American blastomycosis (paracoccidioides brasiliensis)* or Splendore de Almeida Disease is actually the only one of practical importance It is a generalised or localised fungus (Manson) and can also attack the spleen *We are most grateful to Dr Silva of San Paolo for such a spleen* puncture which is reproduced here in Fig 41 *f* The clear strong light refractory spheres with double contours of 1 30 μ in diameter are typical All transitional stages are to be found *i e* from minute configurations containing clear *eccentrically located darkly stained nuclear shaped structures to larger* vitreous spheres (M P da Silva 1943) Moreover the abundance of epithelioid cells some of which fuse to form multinuclear giant cells is further characteristic of the puncture

cate network of the younger nuclei a rather large dark blue nucleolus is present (Figs 37 and 42 *b*) Guzman using special stains in sections has called attention to differences between the nucleoli of these cells and those of normal reticulo endothelium

As the nuclei became older the chromatin becomes closer and thicker (Fig 39) while the nucleoli are usually larger deeper blue and more numerous standing out distinctly in the reddish violet of the nuclear chromatin Increasing segmentation of the nucleus is a further indication of ageing Mitosis without division of the plasma may result in the formation of multi nucleated cells (Figs 38 and 42 *a*)

The cytoplasm is relatively broad being azure blue in the younger types and as maturation increases passing from pale blue to pale violet to grey into the multi nucleated forms in which vacuolation and azurophilic granulation commonly occur

By the examination of many films we found typical mitoses of Dorothy Reed cells (see Fig 42 *f g*) Comparing their chromo somes with those of reticulum cells (Fig 23 *b e f*) the broad cyto plasm of the Dorothy Reed cells and the acute angle of the shorter and clumsier chromosomes is striking Only the basophilic forms seem to enter into division and in these forms in co operation with Thorell we could by using ultra violet absorption microscopy demonstrate large amounts of desoxy ribose nucleic acid This seems to point at the great tendency to proliferation of these young basophilic forms

We doubt the accuracy of Heilmeyer s contention that transitions from reticulo endothelial elements to typical Dorothy Reed cells can be found Equally we are far from convinced that these cells divide by amitosis as Strunge asserts in our opinion it is more probable that the nuclei divide mitotically without concomitant division of the cytoplasm hence the formation of multi nucleated giant cells (see Amitosis p 43)

Our view is that the available evidence strongly suggests the neoplastic nature of these elements and we would specially call attention to the large size of the nucleus relative to that of the cytoplasm the characters of the chromatin the presence of numerous often abnormal nucleoli and the general polymorphism of the cells The local signs of inflammation and the general signs (fever increased B S R) are to be interpreted as the result of a reaction to abnormal protein substances set free by degeneration of the characteristic cells Then again the fact that radio therapy produces an almost specific destructive effect on these cells (Case 4) is further evidence in favour of their neoplastic nature Of course the neoplastic hypothesis does not necessarily exclude a virus as the cause (Grand Castellani) and in a forthcoming publication Schwarz and I will discuss the subject in detail (27 A and 27 B)

We can report on several cases of gland puncture and 8 of spleen puncture

STRUCTURE OF DOROTHY REED (STERNBERG) CELLS IN FILMS

The most striking features are the variations of size of both cell and nucleus and the dark blue or violet nucleolus Probably no other

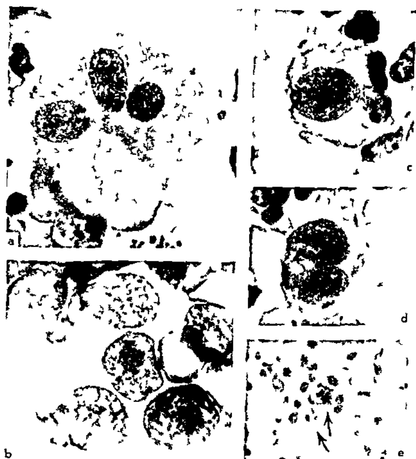


Fig 42 Dorothy Reed (Sternberg) cells in Hodgkin s disease

(a) Multi nucleated forms
(b) Young types
(c) Typical mature cells with finely granular chromatin and dark blue nucleoli
(d) Giant-cell
(e) Histological section from embedded spleen puncture material Two typical Sternberg cells with dark nucleoli are seen

elements except sarcoma and carcinoma cells manifest such differences of size (18–70 μ) In the younger forms the nucleus (14–24 μ) is round or ovoid while in older cells it is up to 51 μ indented loculated or even divided into separate parts which may or may not be connected with one another In the close but deli

In spleen puncture we occasionally saw young macrophages with a bluish nucleolus but these cells showed much smaller nuclei than those of the Sternberg cells The only other conditions in which we have seen the abnormal cell types have been in 2 cases of retothel sarcoma but unlike the condition in Hodgkin s disease there is no associated inflammatory reaction in spleen films which showed mainly lymphocytes with a few neutrophiles mono cytes and plasma cells Indeed in our opinion the absence of a concomitant inflammatory reaction should arouse a suspicion of retothel sarcoma or on other neoplasm

The remarkable size and the basophilia of the nucleoli of Hodgkin cells indicate an intense formation of proteins and may perhaps explain the rapid proliferation of the Hodgkin s tissue The investi gations of Casperssen Thorell and Hyden using ultra violet absorp tion microscopy have shown that normally the precursors of cell proteins are produced by the nucleoli These pass towards the nuclear membrane where it is supposed formation of the cytoplasm occurs Guzman who used a special staining method reached similar conclusions

Spleen Puncture A case in which the diagnosis was made by spleen puncture will be recorded in some detail because the absence of glandular enlargement made clinical diagnosis impossible

Case 1 S S a housewife aged 65 had complained of fatigue anorexia and oppression in the upper abdomen since the beginning of 1935 (aged 59) She was admitted to Professor Clairmont s surgical clinic where an enormous smooth hard swelling of the spleen extending to the navel was discovered as well as a few soft glands the size of peas in the supraclavicular regions Leucocytes 4 200 with neutrophiles 51 per cent lymphocytes 42 5 per cent and no eosinophiles platelets *99 000* red corpuscles *3 8* millions hæmoglobin *78* per cent reticulocytes 1 4 per cent The sternal marrow appeared normal

One of the glands was excised and showed some increase of eosino philes and reticulum cells but no Sternberg cells were found A tentative diagnosis of aleukæmic lymphadenosis was made and radio therapy was started The spleen diminished in size and the general condition improved greatly the patient remaining in good health until 1941

In the spring of that year she was again tired with a temperature of about 100 She again entered hospital in October

Poor general condition Small soft glands the size of peas Liver not enlarged Spleen 13 cm palpable firm and smooth *Blood* hæmoglobin 69 per cent reds 3 7 millions reticulocytes 3 7 per cent occasional normoblasts Leucocytes 2 400 with myelocytes 2 5 per cent staffs 26 per cent polymorphs 22 5 per cent mono- cytes 26 per cent lymphocytes 22 5 per cent no eosinophiles Platelets 105 000 The neutrophile granules were rather coarse Sternal and ilian crest puncture showed slight immaturity of myelopoiesis but no Sternberg cells B S R 2 mm Takata negative Hypoproteinæmia 4 8 mg per cent Prothrombin 50 per cent and no rise after Synkavit Serum iron 120 gamma *Spleen puncture* many typical Hodgkin cells (see Fig 42 *b*) For splenogram see Table 20 Case 1 D ath ensued and



FIG 42 *f g* Typical mitoses ((*f*) prophase (*g*) monaster) of Dorothy Reed Sternberg cells In these mitoses the broad cytoplasm and the acute angle of the shorter and clumsier chromosomes is striking compared with those of reticulum cells (Fig 23 *b e f*)

The typical elements differ from the epithelioid cells of tuberculosis in their possession of bluish violet nucleoli and the blue cytoplasm of the less mature forms The older multi nucleated forms show some resemblance to megakaryocytes (Fig 42 *d*) but can be distinguished by the structure of their nuclei and especially by the characteristic tint of the nucleoli

diagnosis was confirmed by autopsy in the other by the histology of an excised gland

In Case 4 in which the diagnosis had been made histologically spleen puncture performed 6 months after the end of a course of radio therapy failed to reveal any Sternberg cells There were however many eosinophiles (4 6 per cent as compared with the normal 0 2-1 5 per cent) together with 5 per cent of normal mono cytes (see Table 20)

Our other cases also showed some degree of chronic inflammatory change viz distinct monocytosis increase of pulp cells and shift to the left of the neutrophiles Large lymphatic plasma cells were slightly increased but we never found excess of plasmacytoid reticu lum cells Eosinophiles vary from case to case and in different stages of the disease as Ludin also found in gland punctures indeed eosinophiles may be absent

Diagnostic Value of Spleen Puncture Our findings support the views of other writers that spleen puncture can be of great diagnostic aid in Hodgkin s disease In fact if only the spleen is affected and no other means of early diagnosis is available and it may clinch matters in cases in which the histology of excised glands (and gland puncture) gave no clear results

The typical cells are not often found in the sternal marrow (Klima Kienle Paula e Silva) Indeed although sternal puncture is performed as a routine in cases of Hodgkin s disease at the Zurich clinic we have only once been able to demonstrate Dorothy Reed cells This is doubtless due to the fact that involvement of the marrow usually occurs only in the late stages and is even then patchy The spleen on the other hand is often affected very early and puncture is therefore likely to give a positive result

We agree with Tischendorf that the details of Hodgkin s cells are more normally visible in films than in sections of excised glands This is specially important in those early cases in which the histology is simply that of an apparently unspecific over growth of reticulum For this reason if excision of a gland is performed for diagnosis films from the cut surface cells as well as sections should be examined In the former the younger Sternberg cells are more easily dis tinguished from other reticular elements Of course we do not suggest that examination of films should entirely replace sections the two methods supplement one another

D NEOPLASTIC DISEASES OF THE SPLEEN

XI HÆMOBLASTOSES (LEUKÆMIAS)

We cannot discuss the modern use of the pathogenesis of the leukæmias here which has been considered in an earlier work

autopsy (Pathological Institute Professor v Meyenburg) showed Hodgkin s disease in the spleen and liver as well as in the medullary spaces of the pelvic bones and ribs

There was thus a case of intermittent pyrexia with anæmia leucopenia hypoproteinæmia and splenomegaly in which the initial good effect of radio therapy led to suspicion of a neoplastic disease such as a reticulosis or aleukæmic leucosis The low B S R was taken as evidence against a diagnosis of Hodgkin s disease while the hypoproteinæmia was attributed to infiltration of the liver and perhaps of extensive areas of the bone marrow As the spleen puncture showed unexpectedly Dorothy Reed cells the diagnosis was at once cleared

TABLE 20

Hodgkin s Disease

Case number	1	2	3	4
Age sex	65 ♀	26 ♀	49 ♀	39 ♂
Size of spleen (in cm)	13	17	12	
Hæmogram				
Hæmoglobin ( )	69	64	37	—
Leucocytes	2 400	2 200	10 900	—
Neutrophiles staff	26	60¼	16¼	—
Neutrophiles segmented	22½	14¼	16½	—
Eosinophiles	—	½	—	—
Basophiles	½	—	—	—
Monocytes	26	18½	8½	—
Lymphocytes	2[illegible]¼	4½	13¼	—
Plasma cells	—	½	—	—
Splenogram				
Macrophages	0 1	0 2	0 9	—
Plasmacyt retic cells	0 4	0 2	0 1	0 3
Pulp cells	2 1	4 1	0 6	0 7
Erythroblasts	0 2	—	—	—
Myelocytes	1 0	0 6	—	0 3
Neutrophiles staff	6 9	31 6	3 8	7 3
Neutrophiles segmented	6 2	13 [illegible]	13 7	10 7
Eosinophiles	—	1 9	1 1	4 6
Basophiles	0 1	0 2	0 2	—
Monocytes	6 1	9 5	3 3	5 0
Lymphoblasts	—	0 2	—	—
Lymphocytes small young	6 4	1 7	0 6	4 7
Lymphocytes small old	63 8	24 7	70 0	60 0
Lymphocytes large young	2 5	1 0	—	1 3
Lymphocytes large old	[illegible] 4	7 4	0 9	3 7
Lymphocytes total	75 1	35 0	71 5	69 7
Plasma cells	0 5	0 5	0 3	1 4
Total Hodgkin cells	1 3	3 0	4 5	—

In the case of a woman of 26 (Fig 42 *c*) there was leucopenia and anæmia while in a woman of 49 (Fig 42 *a d e*) there was neutrophile leucocytosis and lymphocytopenia In both cases spleen puncture revealed many typical Sternberg cells in one the

phatic leukæmia unless a good deal of blood has become mixed with the spleen fluid

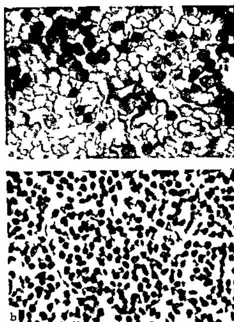


Fig 43 Lymphatic leukæmia (aleukæmic lymphadenosis of the spleen)
(a) Film from spleen puncture purely lymphocytic
(b) Section from spleen puncture

A striking view of spleen films in lymphatic leukæmia is the almost complete absence of pulp cells and a similar state of affairs is found in the lymphosarcoma follicular lymphoblastoma and in some reticuloses Histologically practically no trace of the sinuses can be seen in sections because the proliferating cells practically obliterate them The structural differences between the various cell types are discussed later

Our observations suggest that it is unnecessary for diagnostic purposes to carry out detailed differential count of the cells in spleen films careful examination will give an idea of the approximate distribution and will show the finer details If there is suspicion of lymphatic leukæmia a differential count of 500 cells is advisable and should never be omitted in doubtful cases in which lymphocytes are plentiful in the film

There is one important source of error in such counts it is known that the lymphocytes in blood films in lymphatic leukæmia are structurally delicate which leads to the presence of ruptured cells (Gumprecht shadows) scattered throughout the preparation This is of course an indication of the essentially abnormal character

(Moeschlin and Rohr (3)) in which the leukæmias were discussed as neoplastic diseases

Our observations on spleen puncture in these conditions have been divided into the following sub groups —

(1) Lymphatic leucoses
(2) Myelocytic leucoses
(3) Paramyeloblast leucoses
(4) Polycythæmia vera
(5) Erythroleucoses and erythroblastoses
(6) Reticuloses

Diagnostic Significance Spleen puncture is mainly of diagnostic value in aleukæmic cases (Weil Storti Montes) in which this procedure quite often permits recognition of the disease in most obscure cases *especially of chronic aleukæmic lymphatic leucoses It can also be of diagnostic importance in cases of myeloid reactions in which sternal puncture may not enable a diagnosis to be made either because there is no bone marrow e g in osteosclerosis or because in initial cases no characteristic changes have yet developed in the bone marrow* For instance we have already mentioned (p 61) the case in which well marked clinical inflammatory splenomegaly was accompanied by persistent leucocytosis of 20 000 with a few myeloid cells and only a spleen puncture did permit a definite diagnosis

An interesting sidelight into the details and progress of cases of leukæmia can be obtained by spleen puncture which also permits control of treatment and closer observation of the course of mitosis

1a CHRONIC LYMPHATIC LEUCOSES
(Leukæmia)

Spleen puncture shows great predominance of lymphocytes usually with many immature forms (Weil 1936 Storti Lopez and Montes 1939) *We have investigated 18 cases of which 9 (4 aleu*kæmic and 5 leukæmic) are described here

In all these cases the possibility of paramyeloblastic leukæmia can be excluded because of the clinical course and the characters of the sternal marrow

Lymphocytes in Spleen Puncture

The lymphocytes vary between 92 and 99 per cent which is higher than in any other condition except one case of Felty s disease in which we found 90 per cent of lymphocytes *The presence of more than 95 per cent of lymphocytes therefore permits an almost definite diagnosis of lymphatic leukæmia* If however they are in the region of 90 per cent the characters of the cells and the whole clinical picture must be taken into account Figures below 90 per cent in untreated cases practically exclude the diagnosis of lym

Lymphatic Leukæmias

5	6	7	7	8	9
6- ♀	78 ♂	65 ♀	65 ♀		64 ♀
		(*b*) leukæmic cases			
54	84	83	83	—	88
-47 500	1-5 000	-0 300	13 800	—	?0 100
97	91¼	88¼	90	—	78¼
40 075	114 375	17 9 4	17 4?0	—	15 778
-0	15	27	27	?	11
++	+++	—	—	?	—
—	0 1	—	+	—	—
—	—	—	—	0 5	—
+	+	—	—	—	—
—	—	—	—	0 7	+
0 1	0 1	+	+	—	0 -
0 -	0 5	0 3	0 3	1 2	0 3
0 5	1 9	0 5	0 5	0 7	1 -
—	0 2	—	0 1	0 1	—
—	0 1	—	—	—	0 1
0 3	*0 1*	*0 2*	*0 1*	*0 2*	*0 2*
—	—	—	—	—	—
—	—	—	—	—	—
0 4	1 1	—	—	—	0 8
3 0	6 6	3 0	2 3	11 8	-9 4
93 ?	83 1	94 9	95 8	64 3	65 -
1 4	4 0	0 4	0 2	13 9	1 4
0 9	*2 1*	0 7	0 7	6 6	1 -
98 9	*96 9*	*99 0*	*99 0*	*96 6*	*98 0*
4 4	10 6	3 4	2 5	?5 7	30 8
—	1	—	—	—	1
small immature fragile	extremely polymorphic fragile	small old lymphocytes	uniform fragile	almost normal lymphocytes	mainly young and fragile
43	48	59	65	90	76

seen in normal lymphocytes their occurrence should therefore always arouse a strong suspicion of lymphatic leuk-emia

It is not only the great predominance of lympho-cytes that characterised lymphatic leuk-emia (Fig 43 *a*) the frequency of young cells is striking in most cases (Weil Storti Montes) Norm ally such elements never exceed 10 per cent but a glance at Table 21 reveals their increase in lymphatic leucoses Only in inflammatory diseases and in lymphatic reactions is there a similar increase but of course associated with other indication of inflammation Admittedly *we did not observe any immaturity of the lymphocytes* in 4 cases of lymphatic leuk-emia in which the cells were strikingly small and old

Weil and Montes called attention to the presence of lympho blasts but as our cases show this is inconstant perhaps however they defined lymphoblasts differently On the whole it can be

TABLE 21

Case number	1	2	3	4
Age sex	49 ♀	47 ♀	50 ♂	56 ♂
	(*a*) aleukæmic cases			
Hæmogram				
Hæmoglobin (%)	65	90	?	89
Leucocytes	1 200	2 800	6 900	7 500
Lymphocytes (%)	66½	22	29	51⅓
Lymphocytes absolute	798	616	2 001	3 870
Spleen (in cm)	20	15	16	13
Palpable glands	—	—	+	+
Splenogram				
Macrophages	0 1	—	0 1	+
Plasmacyt retic cells	0 5	+	1 1	+
Pulp cells	0 1	0 1	—	+
Erythroblasts	0 3	—	0 9	—
Myelocytes	—	—	0 1	—
Neutrophiles staff	1 3	1 6	0 5	0 9
Neutrophiles segmented	0 8	4 9	1 8	3 3
Eosinophiles	0 1	0 5	1 0	0 5
Basophiles	—	0 3	0 1	—
Monocytes	1 2	0 5	0 6	0 3
Plasmoblasts	—	—	0 1	—
Plasma cells mature and half mature	0 2	0 1	1 2	0 2
Lymphoblasts	—	0 1	2 4	0 4
Lymphocytes small young	1 1	0 6	19 8	17 0
Lymphocytes small old	86 7	84 4	64 7	67 1
Lymphocytes large young	0 8	0 5	2 9	7 9
Lymphocytes large old	6 8	6 3	2 6	2 4
Lymphocytes total	*95 4*	*91 9*	*92 4*	*94 8*
Lymphocytes total young forms	1 9	1 1	22 7	24 9
Mitosis per 1 000 cells	—	+	3	1
Character of cells	half mature *small* fragile	uniform *very* fragile	very poly*morphic* very fragile	very poly*morphic* very fragile
Puncture number	9	17	57	47 III

of these elements In preparation of spleen films similar shadows are numerous and their presence is suggestive of the diagnosis of lymphatic leukæmia It is therefore essential to choose the best parts of the films for a differential count

Spleen puncture usually supplies plenty of material mixed with very little blood in cases of lymphatic leukæmia and the greyish yellow colour of the fluid immediately arouses suspicion of lymphatic *or chronic myeloid leukæmia or of lymphosarcoma* Sections of embedded puncture material show the gross increase of lymphocytes equally as well (Fig 43 *b*)

Structure These lymphocytes show a striking arrangement of the nuclear chromatin into focal masses (Fig 44 *a*) in almost all cases of lymphatic leucosis Strunge mentions a similar state in the cells of gland puncture (*etat grumelé*) Another common feature is slight lobulation or transverse indentation of the nuclei such as are rarely

morphism and those with many lymphoblasts than in those with predominance of older types of small cells

The other cellular elements in the spleen showed some changes Thus in about half the cases there were isolated erythroblasts and myelocytes but we never found any increase of plasmacytoid reticulum cells In Case 3 there was slight excess of lymphatic plasma cells (1 2 per cent) and also fairly numerous tissue mast cells (0 2 per cent) Unlike the condition found in lymphatic reactions pulp cells are very rarely found in the films

Diagnostic Significance (1) Aleukæmic Cases

Spleen puncture is obviously most likely to be of value for diagnosis in those cases in which there are no glandular swellings and sternal puncture shows no excess of lymphocytes Such cases in which only the spleen and liver are affected at least in the early stages of the disease are by no means uncommon indeed Cohnheim placed them in a group separate from the other lymphadenoses Cases 1 and 2 were of this type and one is recorded here in some detail

Case 1 Housewife aged 49 Complaint of fatigue loss of weight and development of pigmented spots in the extremities Splenic enlargement was detected in 1938 In 1939 eruptions occurred again on the hands and in 1940 she went to the Dermatological Clinic where splenomegaly leucopenia and a positive Wassermann reaction were found She was then referred to the medical clinic

March 7th 1940 poor general condition (45 1 kilos) No enlarged lymphatic glands Liver easily palpable firm and smooth Spleen (20 cm) firm smooth and extending to the navel *Blood picture* hæmoglobin 74 per cent reds 4 4 millions reticulocytes 1 5 per cent leucocytes 1 200 staffs 2 5 per cent polymorphs 16 per cent monocytes 14 5 per cent lymphocytes 66 5 per cent (800 per c mm) *Sternal puncture* slight inhibition of myelopoiesis no percentage increase of lymphocytes nothing suggestive of myeloid leukæmia Histamine fast achylia gastrica B S R 65 W R + + Takata + + Weltmann 0 5 Serum protein 8 5 Prothrombin time 24 seconds (normal 13) Low serum iron (54 gamma) Bilirubin not increased Congo red test for amyloid negative Cholesterol normal (169) Galactose negative Diastase normal Osmotic resistance of reds normal

In brief then this is a case of well marked splenomegaly with leucopenia (1 200) and reduction of lymphocytes to 800 without any enlargement of lymphatic glands The positive Wassermann reaction increased sedimentation rate augmented blood protein level and the achylia aroused a suspicion of syphilitic cirrhosis which is a condition that may be accompanied by great enlargement of the spleen (Naegeli (2)) but the possibility of an aleukæmic leukæmia or some other form of splenomegaly was borne in mind

Sternal puncture showed slight shift to the left to myelopoiesis

said that lymphoblasts are most numerous in cases with cellular polymorphism which we found in only 9 of our 18 cases Variations in size nuclear indentation number and size of nucleoli and breadth of cytoplasm can be seen in the peripheral blood in some cases of lymphatic leukæmia but it is even more striking in spleen films (Fig 44 *a*) in which lymphoblasts also show these peculiarities On the whole cases with cellular polymorphism run a more malignant course than those with predominance of small old lymphocytes



Fig 44 *a* *Lymphatic leukæmia*

(*a*) Variations in size of cells some of young type See striking arrangement of the nuclear chromatin into focal masses

If there is great polymorphism intense basophilia of the cytoplasm delicate nuclear structure with numerous large nucleoli and above all increased blood sedimentation the possibility of lymphosarcoma must be remembered (see p 128)

The cells of reticuloses usually possess closer and more compact chromatin (Fig 51) while those of follicular lymphoblastoma have delicate indentations of the nucleus (Fig 53 *d e*) These features may however be absent and then diagnosis will depend upon the general clinical condition

Our view is that histological diagnosis is even more difficult than cytological because of shrinkage due to fixation

The problems associated with mitosis have already been discussed (p 40) All that need be said here is that cell divisions are more numerous in the cases of lymphatic leukæmia with cellular poly

young basophilic forms (glandular fever) and by the always transient reaction (acute lymphocytosis) The lymphocytes in spleen puncture in these conditions even when the glandular fever cells are included never rise above 85 per cent

The Relationships of Lymphatic Leucosis to Reticulosis

The reticuloses will be discussed in a separate section but it will be convenient to discuss our opinion that many so-called lymphatic leucoses especially the aleukæmic forms should be included among the reticuloses It may be assumed that the neoplastic degeneration in these cases affects some element even less differentiated than the lymphoblast viz. a reticulo-mesenchymal cell But admittedly these elements cannot always be distinguished by their structure from the atypical lymphocytes of lymphatic leucoses Of course if Gitterfasern can be seen there is no doubt as to the diagnosis but in many reticuloses the power of forming fibrillar reticulum has been lost Even so we have reached the conclusion that some cases of aleukæmic or sub-leukæmic lymphatic leucoses (especially those with increased sedimentation rate) are really reticuloses especially because in some cases the increased sedimentation rate is associated with the presence of abnormal plasma globulins or a raised serum protein level similar to the state of affairs in myelomatosis The Takata or cephalin reaction may also be positive in these circumstances while Wuhrmann and Wunderly have demonstrated abnormal electrophoretic curves

In Case 1 the serum protein was 8 5 mg per cent the Takata reaction was positive the blood sedimentation was 61 mm in one hour and there was a probably non specific positive Wassermann reaction In a man of 67 (Case 3) the B S R was 67 mm serum protein 8 5 mg per cent and the Takata reaction was positive In a man of 33 the B S R reached the remarkable figure of 89 mm with a positive Takata reaction and a serum protein level of 9 2 mg per cent (see Fig 50 *d*)

1b ACUTE LYMPHATIC LEUCOSES

Careful consideration of cases in our Clinic has convinced us (Moeschlin and Rohr (3)) that most of the cases recorded under this name in the literature have really been micromyeloblastic For example we have often seen chronic myelocytic leucoses which terminated as micromyeloblastic leukæmias in which the predominant cells were identical with those regarded as typical of acute lymphatic leucosis by Wintrobe (see his Plate XI B p 698)

We would confine the term acute lymphatic leucoses to those cases in which the predominant cells in the blood closely resemble lymphoblasts while in the early stages the disease is confined to the

but no increase of lymphocytes *Spleen puncture (Table 21 Case 1)* revealed the diagnosis immediately because there were 95 4 per cent of lymphocytes typical of an aleukæmic lymphatic leukæmia and quite unlike any inflammatory disease of the spleen Death occurred 6 months later as a result of a lung abscess and the post mortem showed typical lymphadenosis of the liver and spleen but with no glandular enlargement except in the mesentry It seems possible that the Wassermann was probably a non specific result of the changes in the blood proteins

In Case 2 a woman aged 47 there was great enlargement of the liver (23 cm) and distinct splenomegaly (15 cm) without any associated glandular enlargement There was leucopenia (2 800) with only 22 per cent of lymphocytes The serum protein was low (5 9) Takata reaction was negative and the blood sedimentation was low (3 mm) in this case also spleen puncture showed striking fragility of the lymphocytes (Table 21 Case 2) 6 months later there was slight enlargement of some supraclavicular glands

These 2 cases emphasise the difficulty of diagnosis in cases of aleukæmic lymphosclerosis in which there is splenomegaly but no glandular enlargement In Case 1 the association with an infective process increased the difficulty of differential diagnosis and a number of diseases had to be considered We only mention Hodgkin s disease and chronic tuberculosis of the spleen both of which may be impossible to exclude without spleen puncture Diagnosis is of some practical significance because the prognosis in such cases of chronic aleukæmic lymphadenosis is relatively good

In Cases 3 and 4 there was enlargement of lymph glands as well as of the spleen and liver so that the diagnosis could be made by gland puncture In both these cases spleen puncture showed the presence of many young lymphocytes with considerable poly morphism

(2) Sub leukæmic and Leukæmic Types

Spleen puncture is really of little importance in these types of *lymphadenosis because the typical enlargement of lymphatic glands* together with an increase in the absolute numbers of lymphocytes makes a diagnosis obvious except in the rare cases in which only the spleen is involved Then spleen puncture is recommended even if the number of lymphocytes in the peripheral blood is high so as to avoid confusion with lymphatic reaction of non leukæmic nature We have already mentioned such a reaction accompanied by spleno megaly and associated with a pituitary tumour (p 91) In this case a diagnosis was obvious from the character of the spleen films because there was no great degree of lymphocytosis (under 90 per cent) Lymphatic reactions due to infections can be distinguished more easily from lymphatic leukæmias by the appearance of large

cells with broad palely basophilic cytoplasm The nuclei were rounded or elongated with an arrangement of the chromatin closely resembling that of lymphoblasts containing several nucleoli some of which were large (Fig 44 *b*) mitoses were numerous The excessive numbers of young and abnormal cells was unlike the condition found in an ordinary lymphadenosis and a diagnosis of (probable) lymphosarcoma was made

Histologically the spleen structure was almost obliterated and no follicles could be recognised The great cellularity of the pulp led to a suspicion of myeloblastic leukæmia (Pathological Institute University of Zurich Professor von Meyenburg)

The immediate post operative course was satisfactory

Sternal puncture showed normal myelocytic marrow with no pathological cells The *blood picture* was normal for 2 months

A month after the operation a small firm nodule developed in the scar followed by enlargement of the right supraclavicular glands and then by axillary ones Gland puncture revealed cells of the same type as in the earlier spleen films Two months after the operation the leucocytes suddenly rose to 65 800 with 15 200 erythroblasts 2 6 per cent of half mature and mature myelocytes and 60 per cent of lymphatic cells partly of paralymphoblastic type (Fig 44 *c*) There were a few paralymphoblasts with coarse azurophilic granules similar to those of mature lymphocytes

The liver was enlarged Treatment with arsenic and later with urethane caused gradual improvement and decrease of leucocytes but a few paralymphoblasts could still be found in the blood The B S R was persistently low (3–6 mm) There were only a few abnormal cells in the sternal marrow probably due to admixture of blood otherwise erythropoiesis was normal and leucopoiesis was of the ordinary myelocytic type

The patient left hospital at his own wish Large masses of glands developed and death occurred soon afterwards

Summary This was a typical case of lymphosarcoma of the spleen in a man of 50 in whom glandular enlargement and blood changes became manifest 2 months after splenectomy (with about 30 000 per c mm (60 per cent) of paralymphoblasts and paralymphocytes) We were thus able to follow all the developmental stages of a genuine acute lymphatic leukæmia originating in the spleen Thus at first there was an aleukæmic phase with localised lymphosarcoma and then generalisation with involvement of lymphatic glands and probably of the liver together with the appearance of tumour cells in the circulation *i e* the leukæmic stage

The paralymphoblasts showed some signs of a tendency towards maturation into paralymphocytes (Fig 44 *c*) sometimes with the presence of coarse azurophilic granules in the cytoplasm There is thus no doubt of the lymphatic nature of the affected cells and of the close relationship of lymphosarcoma to acute lymphatic leucosis (see also Hauswirth)

It is interesting to find that the bone marrow was not affected until the terminal stage and that urethane had a transiently beneficial effect

spleen and lymphatic glands Of course later the marrow may be affected and diagnosis be impossible In children acute lymphatic leucoses are certainly commoner than in adults (Willi Heilmeyer)

Our conception of acute lymphatic leucosis is thus one of lymphosarcoma with emigration of the abnormal cells into the blood hence the leukæmic blood picture The following case is instructive in this connection —

F A Father died of carcinoma of the œsophagus one brother of an unidentified tumour on the back

Complaint of oppression in left upper abdomen of about a year s duration On November 23rd 1946 sudden and repeated pain in this region was followed by admission to hospital on November 24th 1946 with a tentative diagnosis of acute pancreatitis

There was a firm mass in the upper abdomen with considerable guarding B S R 35 mm Leucocytes 5 600 with 24 per cent of normal lymphocytes Laparotomy was performed because of the prob ability of malignancy but the mass was found to be a greatly enlarged spleen which was removed (1 8 kg)

In films from the spleen there was predominance of large polymorphic



FIG 44 *b c* *Acute lymphatic leukæmia* or perhaps more accurately *leukæmic lymphosarcomatosis*

(*b*) Extremely polymorphous cells with large nucleoli and occasional azure granules (from spleen puncture)

(*c*) Rather more mature types in the blood still showing large nucleoli The cytoplasm of some of the cells shows a pink stain inclusions (↗) in addition to the normal azure granules One normal lymphocyte is present

from 17 per cent to 61 per cent while every type of transitional element between myelocytes and segmented neutrophiles can be recognised (Fig 45) In some cases but by no means in all eosinophile and basophile myelocytes are prominent

In those cases in which the blood contains a very great excess of polynuclears (the leucemie à polynucleaires or Cryptoleucemie of Weil) there are far more immature cells in the spleen than in the blood Incidentally we would cavil at Weil s terminology because in his cases there were up to 20 per cent of myelocytes in the blood

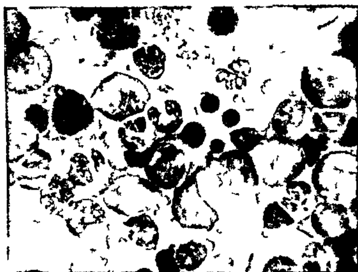


Fig 45 Typical spleen film in chronic myeloid leukæmia with predominance of myelocytes and mature neutrophiles together with many erythroblasts

The proportion of myelocytes to more mature neutrophiles appears to possess prognostic significance Thus cases in which the number of mature granulocytes exceeded that of the myelocytes appeared to run a more chronic course but such a state of affairs may also indicate that the disease is of more recent onset *On the other hand cases in which the number of myelocytes equalled that of more mature granulocytes or exceeded them had a distinctly worse prognosis It is reasonable in such circumstances to assume that the disease has been of longer duration and is approaching its terminal stage* Serial spleen punctures will doubtless throw further light on this subject

Myeloblasts are usually scanty (0 5–1 5 per cent) but higher figures indicate a bad prognosis In all cases in which the myeloblasts were between 2 4 per cent and 17 5 per cent death occurred within a few weeks or at the longest in 9 months Values of 10 per cent or more usually indicate the commencement of a terminal

2 MYELOCYTIC LEUCOSES (CHRONIC MYELOID LEUKÆMIA)

Terminology Before sternal puncture permitted a clear insight into the course of the myeloid leukæmias two types were recognised viz, *chronic* myeloid leukæmia with a blood picture in which myelocytes were numerous together with every form transitional between them and fully mature granulocytes and an *acute* disease in which paramyeloblasts and an "hiatus leukæmicus (Naegeli (1)) were characteristic i e signs of cell maturation were absent We now know that the course of such cases of paramyeloblastic leukæmia may extend over several months or even years (Moeschlin and Rohr (3) Moeschlin (7) Hemmeler) The old distinction into chronic and acute forms is, therefore an erroneous one and we now speak of the former as myelocytic leucoses and of the latter as paramyeloblastic ones It is to be understood that the name "paramyeloblast" is applied to all those abnormal cells, granular or non granular of the myeloid series that manifest only abnormal maturation or none at all

In myelocytic leucosis there is complete disappearance of the normal structure of the spleen as a result of dense infiltration by myelocytes and more mature granulocytes (Lubarsch) Erythroblasts and megakaryocytes can always be found but lymphocytes are scanty and true follicles cannot as a rule be recognised

Spleen puncture confirms the histological picture inasmuch as the predominant cells are myelocytes and elements derived from them so that the films closely resemble those of bone marrow (Nagy 1924 Weil 1936 Storti and Lopez 1939)

Spleen Puncture We have records of 16 cases in which spleen puncture has been performed and in 7 this was done several times in order to follow their course and also the effects of treatment Three cases were erythroleukæmias

The main changes in the blood and spleen are shown in Table 22

As in lymphatic leukæmias spleen puncture supplies plentiful almost bloodless grey marrow like material from which films very rich in cells and resembling marrow can be made Quite often the small lymphoid reticulum cells described in Part I can be recognised among the granular elements whereas in most other spleen films they cannot be distinguished with certainty from the lymphocytes (see Fig 13)

Leucopoietic Series Lymphocytes which form 60 per cent to 85 per cent of the cells in normal and chronic inflammatory spleen films are scanty and may even almost disappear (1 4 per cent in Case 1) while even during a remission due to arsenic urethane or X rays they do rarely rise above 30 per cent (Case 6)

The films are dominated by myelocytes and neutrophiles the former varying from 8 per cent to 51 per cent and the latter

Splenogram (No.)	(68)	(107)	(94)	(1)	(70)	(8)	(116)	(118)	(71)
Macrophages	—	—	0.1	0.5	—	+	0.1	+	+
Plasmacyt. retic. cells	—	0.2	—	0.1	0.3	0.3	0.3	1.1	—
Pulp cells	—	—	—	0.2	—	0.1	—	0.1	—
Erythroblasts									
Total	6.6	4.8	7.2	1.0	7.4	1.1	13.4	15.8	2.1
Basophilic	0.6	0.8	1	0.1	0.3	0.3	1.5	2.9	0.3
Polychromatic	3.1	3.6	5.0	0.4	4.5	0.8	8.7	11.7	0.8
Orthochromatic	2.9	0.4	1.0	0.5	2.6	—	3.2	1.2	1.0
Myeloblasts	2.4	1.7	2.4	0.2	0.4	—	0.5	0.2	17.5
Myelocytes total	51.2	50.1	34.4	37.1	37.6	8.4	2.9	10.9	39.7
Myelocytes immature									
Neutrophile	10.9	6.8	1.4	1.0	3.6	1.0	1.1	1.0	4.7
Eosinophile	0.1	0.2	0.1	—	0.5	—	0.2	—	—
Basophile	—	—	—	—	—	—	—	—	—
Myelocytes ½ mature									
Neutrophile	21.8	15.5	9.9	9.1	6.7	[illegible]8	3.8	1.8	14.9
Eosinophile	0.[illegible]	0.4	0.5	—	0.6	—	0.1	0.8	—
Basophile	—	0.1	—	—	—	—	—	—	—
Myelocytes mature									
Neutrophile	3.0	10.7	9.3	3.8	9.1	[illegible]2	5.2	3.3	6.9
Eosinophile	0.6	0.6	—	—	1.9	0.1	0.3	0.1	0.6
Basophile	—	0.1	—	—	—	—	—	—	—
Metamyelocytes	14.6	15.7	13.2	18.2	15.2	3	12.2	3.9	12.6
Myelocytes—mitoses (per 1 000)	2	1	2	—	2	—	1	1	2
Neutrophiles									
Staff	22.4	23.6	2[illegible].6	36.9	9.6	16.0	[illegible]5.1	1[illegible].1	2.8
Segmented	10.4	6.6	15.0	[illegible]2.9	8.8	44.9	12.0	14.9	10.5
Eosinophiles mature	1.1	0.6	3.5	0.2	1.7	0.2	2.2	6.3	—
Basophiles mature	4.0	3.3	3.6	0.6	2.6	6.0	3.0	4.7	2.6
Monocytes	0.5	1.1	0.8	1.7	0.1	4.0	0.8	2.4	0.1
Lymphocytes total	1.4	8.0	10.4	3.4	11.5	18.9	19.7	31.5	4.7
Plasma cells	—	—	—	0.1	—	0.1	—	—	—
Megakaryocytes	+	+	+	0.1	+	+	+	—	—

Splenogram (No.)	(12)	(130)	(99)	(18)	(72)	(97)	(104)	(108)	(145)
Macrophages	+	—	—	—	0.1	—	—	0.1	—
Plasmacyt. retic. cells	0.[illegible]	0.2	—	—	0.1	0.3	+	1.2	0.1
Pulp cells	—	0.2	—	—	—	—	0.2	0.8	0.1
Erythroblasts									
Total	11.8	45.3	13.2	15.2	33.2	59.2	45.0	40.8	53.5
Basophilic	1.4	4.1	0.8	10.0	[illegible]2.9	17.4	9.5	9.6	5.3
Polychromatic	6.9	4.8	9.9	4.8	6.5	34.7	32.1	9.4	44.1
Orthochromatic	3.5	16.4	2.5	0.4	3.8	7.1	3.4	1.8	4.1
Myeloblasts	1.7	0.7	4.3	2.1	4.6	4.3	3.3	1.4	—
Myelocytes total	37.4	8.5	45.3	31.7	22.1	11.1	8.0	12.6	5.2
Myelocytes immature									
Neutrophile	6.6	0.4	6.6	3.0	4.1	[illegible]7	1.6	2.1	0.8
Eosinophile	—	—	0.1	0.6	—	0.1	0.3	—	—
Basophile	—	—	—	0.1	—	—	—	—	—
Myelocytes ½ mature									
Neutrophile	9.2	1.8	8.3	5.8	4.8	2.9	2.8	[illegible]8	1.9
Eosinophile	—	0.3	1.0	[illegible]3	0.5	1.5	0.7	0.2	—
Basophile	—	0.1	—	0.4	0.7	—	—	—	0.1
Myelocytes mature									
Neutrophile	8.9	1.6	15.0	4.0	2.8	2.2	0.6	2.5	1.4
Eosinophile	0.2	0.2	2.3	2.5	1.2	0.8	0.9	0.4	—
Basophile	—	1.5	—	6.1	3.0	0.2	0.1	0.[illegible]	0.1
Metamyelocytes	12.5	[illegible]6	12.0	6.9	5.0	0.7	1.0	4.4	0.9
Myelocytes—mitoses (per 1 000)	2	—	1	—	—	—	—	1	—
Neutrophiles									
Staff	23.2	8.4	10.8	18.2	13.[illegible]	[illegible]8	6.0	9.1	4.2
Segmented	11.8	11.1	6.0	11.1	8.7	1.8	5.2	6.0	8.9
Eosinophiles mature	1.3	1.4	3.9	5.6	1.6	1.8	7.3	0.9	—
Basophiles mature	[illegible]4	1.6	8.2	9.0	9.1	9.2	9.1	0.8	0.2
Monocytes	0.2	0.8	—	—	0.1	—	0.4	1.7	0.2
Lymphocytes total	10.0	21.8	8.3	7.1	7.1	9.5	15.4	24.6	[illegible]7.5
Plasma cells	—	—	—	—	—	—	0.1	—	—
Megakaryocytes	—	+	+	—	0.1	+	—	+	0.1

TABLE 22

Chronic Myeloses

Case number	1	2	3	4	5 (a)	5 (b)	6 (a)	6 (b)	7	8 (a)	8 (b)	9	10 (a)	10 (b)	11 (a)	11 (b)	12	13
Age	64	34	34	60	48		34		75	66		44	33		42		61	54
Sex	♂	o	o	o	o		♂		♀	♀		♀	♀		♀		♀	♂
Duration of symptoms (in years)	3	1½	3	1?	2		½		5	3½		2	4		1–2		6	2–5 ?
Size of spleen (in cm)	23	20	13	15	8		15	12	33	25		34		27	17	24	20	42
Hæmogram	11/9/1942	6/6/1944	9/9/1943	4/9/1936	12/7/1940	26/11/1940	2/11/1944	16/11/1944	30/7/1940	28/3/1940	23/2/1945	26/2/1944	9/5/1940	9/9/1940	29/12/1943	22/3/1944	3/5/1944	27/2/1946
Red corpuscles (in mills.)	3 260	4 440	4 850	1 836	3 860	4 750	5 230	4 456	—	4 220	4 660	2 840	1 490	1 960	—	3 140	3 670	6 200
Erythroblasts (per 100 leucocytes)	11	—	—	—			2	—	1½	1½	1½	rarely	18	14	3	22½	6	60
Hæmoglobin ( )	53	91	101	31	—	—	90	91	62	80	78	60	27	34	77	64	67	118
Leucocytes (in 1 000s)	574 4	96 9	80 2	426 4	144 0	15 5	81 2	11 5	400 0	112 8	8 4	151 2	37 2	27 8	6 6	13 4	10 4	15 0
Myeloblasts	3	⅓	⅓	1⅓	—	—	—	—	17½	½	—	4	3	11	6	3⅓	2½	(1)
Myelocytes																		
Total	57⅔	24	23	16	15	4⅔	16	2½	28⅓	24	3	33	14	17	11	6	10½	4½
Immature	18	4	3	1½	—	—	4⅓	—	5⅓	3½	—	9	5⅔	5⅓	4½	2	1	2½
Half mature	24	7	14⅓	2½	—	—	2⅔	1	11	9	1	16½	4⅓	4	3½	3	2½	1
Mature	4⅔	2⅓	1⅔	½	—	—	5	—	7½	1	—	3½	⅔	1⅓	1	⅓	3	—
Metamyelocytes	11	10⅔	4	11½	—	—	4	1⅓	4½	10½	2	4	3	6⅓	2	⅔	4	1
Neutrophiles																		
Staff	25	48½	49⅔	15½	55	34⅓	41	20	40½	52⅓	20	23½	29⅓	19	17	18⅓	52½	27½
Segmented	4⅓	16	20⅔	62½	21	45⅔	31⅔	48⅓	11⅓	15	60	20	10⅔	18⅔	16	20⅔	20	59
Basophiles (myelocytes and mature)	5⅔	4	2⅓	½	6⅓	6⅔	3⅓	3½	1½	2½	5	13½	28½	26	29½	30	3½	6½
Eosinophiles (myelocytes and mature)	4	—	1⅔	—	1	—	1⅔	7½	¼	⅓	2	3½	11	5	4	11	1	—
Monocytes	⅓	3⅔	1	2	1	5⅓	2⅓	6½	—	2	1	1	—	—	½	—	6½	½
Lymphocytes	—	3⅔	1	2	½	3⅓	4	11½	—	3	9	1½	2⅔	3⅓	15½	10⅔	3½	6½

films is irregular perhaps because their large size interferes with their being separated from their connections If a sufficient amount of material is obtained for the preparation of histological sections it is easy to see how much more numerous these cells are than in the case of films (Fig 46 *b*) It is as equally true of the sternal marrow in which megakaryocytes are approximately 10 times more numerous in sections than in films (Moeschlin (6)) Such cells may be ex tremely numerous in the so-called megakaryocytic leucoses (see later)

The Cause of Occurrence of Erythroblasts and Megakaryocytes Most authors such as Naegeli Forkner Apitz and others assume that all the cellular systems of myeloid tissue are involved in chronic myelocytic leukæmia Formerly we have suggested the possibility that the erythroblasts and megakaryocytes which appear in the spleen and other organs in myelocytic leucoses arise as a compen satory phenomenon which to some extent makes good the damage to erythropoiesis and platelet formation due to compression by proliferating granulocytes in the marrow This state of affairs is well seen in paramyeloblastic leucoses where oxydase negative paramyeloblasts can be seen lying close to typical erythroblasts and oxydase positive myelocytes in the liver and spleen (Moesch lin (1)) Such a proof is not possible in myelocytic leucoses because all the cells are oxydase positive

The occurrence of chronic myelocytic leucoses in which the forma tion of megakaryocytes is particularly striking (the so called mega karyocytic leukæmias described by Downey Nordland McDonald and others) and also of erythroblastic leukæmias (in which erythro blasts are greatly increased in the marrow also) strongly suggests *that in the chronic leukæmias it is not only the granulocytic series but the whole myeloid system that is affected and that the differences between cases depend upon the intensity of the proliferation of one or other of the cell series*

Differences between the Myeloid Composition of the Spleen and Bone Marrow Table 23 presents the splenograms and myelograms of 5 untreated cases in which the results of the sternal punctures have been given in percentages in order to make the values directly comparable The sternal marrow is generally found to contain more immature myelocytes than the spleen which contains more mature myelocytes and metamyelocytes than the marrow *That is to say the bone marrow in chronic myelocytic leucoses shows a greater degree of shift to the left than does the myeloid tissue of the spleen* The number of myeloblasts in the two organs shows no constant differ ence and indeed in some cases these cells were rather more numerous than in the spleen The total number of myelocytes shows varia tions sometimes being greater in the marrow sometimes in the spleen while erythroblasts also show no constant relationship

paramyeloblastic aggravation which will be usually refractory to treatment and will lead to death in a few weeks or months

The structural characters of leukæmic cells have been discussed elsewhere (3) in collaboration with Rohr In spleen films the myelocytes show the same types of abnormality of structure and development as in the blood and marrow Thus there is dissociation of maturation of nucleus and cytoplasm and persistence of nucleoli sometimes even when the nucleus has become indented On the other hand the granules may be very immature and remain clotted together at a time when the nucleus is already mature

Erythroblasts As shown in the table erythroblasts are always found in spleen films In myelocytic leucoses polychromatic forms

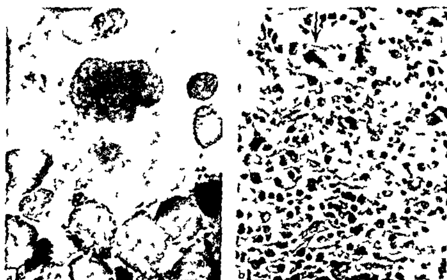


FIG 46 Megakaryocytes in the spleen in chronic myeloid leucoses
(a) Film (b) Section

are much more numerous than are basophilic and oxyphilic ones while occasionally large basophilic pro erythroblasts are found Mitoses are commonest among the polychromatic types Cases in which the erythroblasts are as high as 13 per cent or occasionally 33 per cent (Case 10) are not very uncommon and cells of the erythropoietic series may be more numerous than in marrow films Such an excess of erythroblasts in spleen puncture is thus a regular feature of myelocytic leucoses but one should not regard a case as being an erythroleukæmia unless the erythroblasts are also greatly increased in the marrow

Megakaryocytes These cells can be found in the spleen in almost all cases of myelocytic leucosis (Fig 46 a) We were unable to find them in only 2 out of 16 cases The distribution of the cells in

films is irregular perhaps because their large size interferes with their being separated from their connections If a sufficient amount of material is obtained for the preparation of histological sections it is easy to see how much more numerous these cells are than in the case of films (Fig 46 *b*) It is as equally true of the sternal marrow in which megakaryocytes are approximately 10 times more numerous in sections than in films (Moeschlin (6)) Such cells may be extremely numerous in the so called megakaryocytic leucoses (see later)

The Cause of Occurrence of Erythroblasts and Megakaryocytes Most authors such as Naegeli Forkner Apitz and others assume that all the cellular systems of myeloid tissue are involved in chronic myelocytic leukæmia Formerly we have suggested the possibility that the erythroblasts and megakaryocytes which appear in the spleen and other organs in myelocytic leucoses arise as a compensatory phenomenon which to some extent makes good the damage to erythropoiesis and platelet formation due to compression by proliferating granulocytes in the marrow This state of affairs is well seen in paramyeloblastic leucoses where oxydase negative paramyeloblasts can be seen lying close to typical erythroblasts and oxydase positive myelocytes in the liver and spleen (Moeschlin (1)) Such a proof is not possible in myelocytic leucoses because all the cells are oxydase positive

The occurrence of chronic myelocytic leucoses in which the formation of megakaryocytes is particularly striking (the so called megakaryocytic leukæmias described by Downey Nordland McDonald and others) and also of erythroblastic leukæmias (in which erythroblasts are greatly increased in the marrow also) strongly suggests *that in the chronic leukæmias it is not only the granulocytic series but the whole myeloid system that is affected and that the differences between cases depend upon the intensity of the proliferation of one or other of the cell series*

Differences between the Myeloid Composition of the Spleen and Bone Marrow Table 23 presents the splenograms and myelograms of 5 untreated cases in which the results of the sternal punctures have been given in percentages in order to make the values directly comparable The sternal marrow is generally found to contain more immature myelocytes than the spleen which contains more mature myelocytes and metamyelocytes than the marrow *That is to say the bone marrow in chronic myelocytic leucoses shows a greater degree of shift to the left than does the myeloid tissue of the spleen* The number of myeloblasts in the two organs shows no constant difference and indeed in some cases these cells were rather more numerous than in the spleen The total number of myelocytes shows variations sometimes being greater in the marrow sometimes in the spleen while erythroblasts also show no constant relationship

paramyeloblastic aggravation which will be usually refractory to treatment and will lead to death in a few weeks or months

The structural characters of leukæmic cells have been discussed elsewhere (3), in collaboration with Rohr In spleen films the myelocytes show the same types of abnormality of structure and development as in the blood and marrow Thus there is dissociation of maturation of nucleus and cytoplasm, and persistence of nucleoli sometimes even when the nucleus has become indented On the other hand the granules may be very immature and remain clotted together at a time when the nucleus is already mature

Erythroblasts As shown in the table erythroblasts are always found in spleen films In myelocytic leucoses polychromatic forms

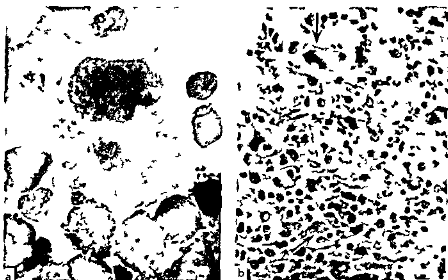


FIG 46 Megakaryocytes in the spleen in chronic myeloid leucoses
(a) Film (b) Section

are much more numerous than are basophilic and oxyphilic ones while occasionally large basophilic pro erythroblasts are found Mitoses are commonest among the polychromatic types Cases in which the erythroblasts are as high as 13 per cent or occasionally 33 per cent (Case 10) are not very uncommon and cells of the erythropoietic series may be more numerous than in marrow films Such an excess of erythroblasts in spleen puncture is thus a regular feature of myelocytic leucoses but one should not regard a case as being an erythroleukæmia unless the erythroblasts are also greatly increased in the marrow

Megakaryocytes These cells can be found in the spleen in almost all cases of myelocytic leucosis (Fig 46 *a*) We were unable to find them in only 2 out of 16 cases The distribution of the cells in

by the spleen such as occurs in other examples of hypersplenism but the presence of numerous mitoses seems incompatible with this In the present state of our knowledge it is impossible to ascertain what other factors play a part

Tropeano has recently confirmed our findings In 10 cases of leucoses (8 chronic and 2 acute) he found that erythropoiesis was more active in the spleen than in the marrow Also he states that he found no differences *in the granulocytes in the two organs If however one examines his* myelograms and splenograms carefully one finds that (except in Cases 6 and 7) there is a larger number of immature forms (myeloblasts and pre myeolocytes) in the marrow than in the spleen Case 7 is to be excluded because the immature cells were overshadowed by the great proliferation *of reticulo endothelial nature (2 4 per cent) It is also possible that in* his Case 6 in which there were more immature cells in the spleen than in the marrow he was really dealing with the terminal stage of a leucosis in which there was a gradual acute dedifferentiation beginning

Myelograms in Chronic Myeloid Leukæmia
and mustard gas for further cases)

Granulocytic series												
	Myelocytes					Neutrophiles			Mature			
Myeloblasts	Immature	Half mature	Mature	Metamyelocytes	Total	Staff	Segmented	Total	Eosinophiles	Basophiles	Monocytes	Lymphocytes Total
2 4	11 0	22 0	3 6	14 6	51 2	2? 4	10 4	32 8	1 1	4 0	0 5	1 4
	33 0		18 2									
1 5	13 6	30 8	5 0	9 7	59 1	?6 9	4 6	31 5	1 4	1 9	0 5	0 4
	44 4		14 7									
1 7	7 0	16 0	11 4	15 7	?0 1	23 6	6 6	30 2	0 6	3 3	1 1	8 0
	23 0		27 1									
0 4	9 4	22 6	4 5	9 7	46 2	33 7	13 4	47 1	1 8	1 5	0 4	1 4
	32 0		14 2									
0 5	1 3	3 9	5 5	12 2	2? 9	25 1	12 0	37 1	2 2	3 0	0 8	19 7
	5 2		17 7									
1 0	10 4	32 2	6 0	8 0	56 6	23 0	6 8	?9 8	3 0	1 2	0 6	1 0
	42 6		14 0									
4 3	6 7	9 3	17 3	12 0	45 3	10 8	6 0	16 8	3 9	8 2	—	8 3
	16 0		29 3									
3 3	5 4	0 3	11 4	10 2	47 3	?4 5	13 6	38 1	3 6	5 5	—	1 0
	25 7		?1 6									
? 1	3 7	8 5	12 6	6 9	31 7	18 2	11 1	29 3	5 6	9 0	—	7 1
	1? 2		19 5									
7 ?	4 2	14 5	8 ?	5 7	3? 6	15 9	7 4	23 3	3 6	2 7	—	9 0
	18 7		13 9									

It was however found that erythroblasts were always more numerous in the spleen than in marrow films This is certainly not due to admixture of blood because in all our cases the spleen juice obtained by puncture was much less bloody than that from the marrow Further, as mentioned below, the shift to the left of the myelogram as compared with the splenogram persists after treatment with arsenic and X rays *i e*, after the blood picture has more or less returned to normal (Table 27)

Enumeration of the mitoses shows that the excessive number of immature forms in the marrow is accompanied by more numerous cell divisions *There can therefore be no doubt about these differences between the spleen and marrow in the cellular composition*

What may be the Cause of this Difference? The first possibility to occur to one is that there is a hormonal inhibition of the marrow

TABLE 23 *Simultaneous Splenograms and*
(See chapter on urethane arsenic

Case number	Age sex		Erythroblasts Basophile	Erythroblasts Polychromatic	Erythroblasts Orthochromatic	Erythroblasts Total
1	64 ♂ (68)	Spleen	0 6	3 1	2 9	6 6
		Marrow	0 6	2 4	0 7	3 7
2	34 ♀ (116)	Spleen	0 8	3 6	0 4	4 8
		Marrow	0 1	1 0	—	1 1
6	34 ♂ (107)	Spleen	1 5	8 7	3 2	13 4
		Marrow	1 0	4 8	0 9	6 7
9	44 ♂ (99)	Spleen	0 8	9 9	[illegible] 5	13 2
		Marrow	0 1	0 7	—	0 8
10	33 ♀ (18)	Spleen	10 0	4 8	0 4	15 [illegible]
		Marrow	9 5	10 9	1 0	[illegible]1 4

TABLE 24

Number and Distribution of Mitoses found by Spleen and Marrow Punctures in Chronic Myelocytic Leucoses

Case 2 (male aged 34)	Prophase	Monaster	Diaster	Dispireme	Total cells in mitosis	Number of cells per 1 mitosis	Mitoses per 1 000
I Spleen							
Myeloblasts	—	—	—	—	0	855	0
Myelocytes immature	1	7	2	—	10	3 5 3	2 8
Myelocytes half mature	1	18	5	—	24	8 054	2 9
Myelocytes mature and meta	—	6	—	—	6	13 642	0 4
Mature granulocytes	—	—	—	—	0	17 7 2	0
Total	2	31	7	0	40	43 796	
Per 100 mitos forms	5	77 5	17 5	0	100	109 490	
Mitotic index							0 91
II Sternum							
Myeloblasts	—	—	—	—	0	77	0
Myelocytes immature	1	7	2	—	10	1 818	5 5
Myelocytes half mature	3	19	2	2	6	4 371	5 9
Myelocytes mature and meta	—	3	1	—	4	4 746	1 4
Mature granulocytes	—	—	—	—	0	10 857	0
Total	4	29	5	2	40	18 842	
Per 100 mitos forms	10	72 5	12 5	5	100	47 105	
Mitotic index							2 12

Case 1 (male aged 64)	Prophase	Monaster	Diaster	Dispireme	Total cells in mitosis	Number of cells per 1 mitosis	Mitoses per 1 000
I Spleen							
Myeloblasts	—	—	—	—	0	1 178	0
Myelocytes immature	5	9	4	—	18	5 171	3 4
Myelocytes half mature	5	9	2	1	17	10 343	1 6
Myelocytes mature and meta	—	4	1	—	5	8 556	0 5
Mature granulocytes	—	—	—	—	0	18 056	0
Total	10	2	7	1	40	43 754	
Per 100 mitos forms	25	55	17 5	2 5	100	108 779	
Mitotic index							0 92
II Sternum							
Myeloblasts	—	—	1	—	1	07	0 5
Myelocytes immature	6	2	2	—	10	1 836	5 4
Myelocytes half mature	11	6	—	—	17	4 160	4 8
Myelocytes mature and meta	4	7	1	—	12	1 985	6 0
Mature granulocytes	—	—	—	—	0	4 770	0
Total	21	15	4	0	40	1 953	
Per 100 mitos forms	52 5	37 5	10	0	100	3 382	
Mitotic index							3 08

In only 1 case, which had been treated with urethane we found a larger number of immature cells in the spleen than in the marrow At this time the blood picture showed no signs of an imminent acute exacerbation but the condition in the spleen suggested that this would occur shortly and in fact it did so 2 weeks later As Rohr has pointed out such terminal conversion of chronic myeloid leukæmia into acute paramyeloblastic leucosis often commences in the spleen at a time when the bone marrow is relatively unaltered We have however recently seen a case in which the terminal differentiation started in the marrow and not in the spleen *Our present view is that the presence of more immature granulocytes in the spleen than in the marrow is to be regarded as being indicative of an approaching terminal phase*

DIFFERENCES IN THE FREQUENCY AND DISTRIBUTION OF MITOSES IN THE SPLEEN AND BONE MARROW

Only an outline of our observations will be given here as we intend to publish them in detail later In the spleen, as in the marrow in chronic myelocytic leucosis one finds numerous mitoses in every type of cell from the myeloblasts to the myelocytes and metamyelocytes In order to obtain a quantitative assessment of the individual stages of mitosis and their distribution among the various types of granulocytes we have performed serial examinations of the sternal marrow and the spleen in 2 untreated cases The results will be found in Table 24

We have been able to confirm Fieschi s statement that the percentage values of mitoses in the sternal marrow in chronic myelocytic leukæmia are approximately the same as those of mitoses in normal granulocytes in the marrow Thus Rohr in two normal persons found a mitotic index of 1 8–2 5 per 1 000 whereas in our 2 cases of leukæmia the values in the sternal marrow were 3–3 6 per 1 000 respectively The normal values given by other writers also lie within these limits thus Picena gives 1–2 per 1 000 Fieschi 1 per 1 000 with variations up to 5 or 6 per 1 000 and Weerdt 2 9 per 1 000 *The mitotic index in the marrow in chronic myelocytic leukæmia is thus only slightly above normal* i e *the relative number of mitoses is only slightly increased but naturally the total number is greatly raised because of the enormous extension of active leukæmic marrow tissue*

The state of granulocyte mitoses in the spleen as compared with that in the sternal marrow is very surprising Thus in both our cases there were strikingly fewer mitoses in the spleen than in the marrow *Marrow films on the average show a three or four times higher mitotic index than do spleen films* At present we can scarcely do more than guess at the significance of these findings Neither of our cases had received any treatment so that no external factor

doses (2 500–3 000 r) complete aplasia of the marrow occurred which however was followed by slight regeneration

The literature does not contain any detailed account of the effect of X irradiation of the bone marrow in chronic myelocytic leucoses (except 1 case recorded by Stephens) or on the extramedullary myeloid tissue of the spleen In 1 case we punctured the spleen 24 hours after 120 r had been given and found striking fragility of the cells together with many degenerate myelocytes some of which showed nuclear pyknosis and formation of vacuoles in the cytoplasm *There were many macrophages but the erythroblasts showed no* structural changes and seemed to be slightly more numerous than in the previous puncture The fragility of the cells was so great that a detailed qualitative assessment was impossible and for the same reason it was impossible to judge of the frequency of mitoses In the following case the changes in the spleen and marrow were

TABLE 25

	Before irradiation			Four months after irradiation		
	Hæmogram	Splenogram	Myelogram	Hæmogram	Splenogram	Myelogram
Total leucocytes (in 1 000 s)	144 0	—	—	15 5	—	—
Myeloblasts	—	—	—	—	—	—
Myelocytes immature	—	—	—	—	—	—
Myelocytes half mature	—	—	—	—	—	—
Myelocytes mature	—	—	—	—	—	—
Metamyelocytes	—	—	—	—	—	—
Total myelocytes	15	—	—	4⅔	—	—
Neutrophiles staff	55	—	—	45⅔	—	—
Neutrophiles segmented	21	—	—	34⅓	—	—
Eosinophiles (myelocytes and mature)	1	—	—	—	—	—
Basophiles (myelocytes and mature)	6½	—	—	6⅓	—	—
Monocytes	1	—	—	5⅓	—	—
Lymphocytes	½	—	—	3⅓	—	—
Macrophages	—	—	—	—	+	—
Plasmacyt reti cells	—	0 3	—	—	0 3	—
Pulp cells	—	—	—	—	0 1	—
Erythroblasts basophile	—	0 3	1 0	—	0 3	4 0
Erythroblasts polychrom	—	4 5	1 3	—	0 8	10 7
Erythroblasts orthochrom	—	⸤ 6	1 0	—	—	0 9
Erythroblasts total	—	7 4	3 3	—	1 1	15 6
Myeloblasts	—	0 4	0 4	—	—	0 3
Myelocytes immature	—	4 1	4 2	—	1 0	6 6
Myelocytes half mature	—	7 3	13 0	—	2 8	10 9
Myelocytes mature	—	11 0	13 2	—	? 3	14 6
Metamyelocytes	—	15 2	7 3	—	2 3	14 2
Total myelocytes	—	37 6	37 7	—	8 4	46 3
Neutrophiles staff	—	29 6	40 1	—	16 0	6 5
Neutrophiles segmented	—	8 8	11 2	—	44 9	6 8
Eosinophiles mature	—	1 7	4 3	—	0 2	1 5
Basophiles mature	—	2 6	1 9	—	6 0	0 9
Monocytes	—	0 1	0 1	—	4 0	0 3
Total lymphocytes	—	11 5	1 0	—	18 9	1 8
Megakaryocytes	—	+	+	—	+	+

can be responsible for the appearances Even in the cases discussed below the mitotic index in the sternal marrow was three times higher than that in the spleen As far as one may base conclusions on only 6 cases this would seem to be an invariable difference between the myeloid tissue of the spleen and marrow in chronic myeloid leukæmias It is certain that the reduction of the mitotic index is not due to admixture of blood with the spleen juice because none of our films contained much blood The speed of mitoses in myelocytes in the marrow and spleen is not likely to be different but it is conceivable that there might be a hormonal inhibition of mitosis in the marrow which would prolong it and thus account for the greater number of cell divisions, but at present little is known about the reciprocal relations of the spleen and marrow Splenectomy in leukæmias appears to exert an unfavourable influence in chronic myelocytic leukæmia (Forkner) leading to rapid deterioration

If the percentage distribution of cells in marrow and spleen films is compared one finds distinctly more mature forms in the latter (Table 23) This seems to indicate that there is some kind of hormonal influence in the sense of the increase of immature forms in the marrow and possibility of delay in the maturation of myeloid cells If this were so an increased number of mitoses in the marrow might well be the result of the presence of numerous primitive cells, which in the spleen also show more mitoses than do the older types This explanation is admittedly not entirely satisfactory

If we examine the distribution of mitoses in the various stages of cell development we find that in the spleen the mitoses are fairly evenly distributed between the immature and the half mature myelocytes 2–3 per 1 000 whereas in spite of their great number mitoses in the more mature cells (mature myelocytes and metamyelocytes) are scantier (Table 24) *In chronic myelocytic leucoses therefore the myelocytes are to be regarded as maturing elements while new formation of daughter cells occurs by mitotic division of the immature and half mature forms* This formative activity of the immature forms is even more noticeable in the sternal marrow Thus in Case 2 6 per 1 000 of the immature myelocytes and 5 per 1 000 of the half mature ones were in mitosis (Table 24)

THE EFFECTS OF X RAYS ON THE SPLEEN AND MARROW

Rachmilewitz (1947) who investigated the radio sensitivity of cultures of normal marrow cells observed decrease in the number of myelocytes and a greater sensitivity of the more mature forms of both granulocytes and erythroblasts Denstad observed decrease of red and white cells after irradiation of the sternum with 300 r but even then the erythroblasts were rather more sensitive With larger

examined before X ray treatment and 4 months after it, the total doses given over the splenic area being 800 r The total number of leucocytes corresponding to 20 mitoses was counted on both occasions (Table 25 and Fig 46 c)

This example demonstrates that the therapeutic effect of X rays does not depend upon a general influence but purely upon the action of irradiation on the myeloid tissue in the treated area Rays cause three types of changes in the affected myeloid tissue (1) A distinct reduction in the amount of myeloid tissue in the spleen, this is both absolute as shown by decrease in size of the organ and also relative inasmuch as there is a percentage decrease of granulocytes and in consequence, a relative rise of lymphocytes (2) There is a greater reduction of the most immature types associated with a relative increase of the mature ones this may well be the most important therapeutic effect of the treatment In this case spleen puncture before irradiation showed 38 per cent of immature cells but only 8 per cent after treatment This is probably attributable to a greater degree of sensitivity of the more immature cells Certainly it is improbable that irradiation causes accelerated maturation as Isaacs thought The number of erythroblasts also falls after irradiation (3) In the directly irradiated area there is an absolute and relative decrease of mitoses probably as a result of destruction of the most immature elements Thus before irradiation the mitotic index in the spleen was 1 0 per 1 000 and after the irradiation 0 1 per 1 000 *i e* a decrease to a tenth part of the original value (Table 26 and Fig 46 c)

It is striking that the sternal marrow and also the remainder of the bone marrow except that in the area of irradiation *still show typical leukæmic changes with excess of immature forms and many mitoses even after a complete course of radio therapy in fact the typical changes may become more marked* A comparison of the blood picture with the myelogram shows that the increase in the number of mitoses in the marrow cannot be attributed to diminished admixture of granulocytes from the blood If such increase of mitoses in the bone marrow after irradiation of the spleen can be confirmed in other cases of chronic myelocytic leucosis we should have a reasonable explanation of the decrease of hormonal inhibition of the marrow which follows diminution in size of the spleen The only observation which we are aware is that of Stephens who observed 1 case in which 500 r were given over the thorax and spleen 5 months earlier He found that in the sternal marrow immature myeloid cells had fallen from 52 7 to 24 3 per cent Biopsy from the sternum before irradiation of the thorax showed increased hyperplasia of the myeloid tissue whereas 5 months later the marrow was poor in cells and fibrotic This case is not directly comparable with ours because the sternal marrow as well as the

TABLE 26

Number and Distribution of Mitoses in Spleen and Sternal Puncture Before and After X irradiation

Case 5 (male aged 48)

	Spleen							Marrow						
	Prophase	Monaster	Diaster	Dispireme	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000	Prophase	Monaster	Diaster	Dispireme	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000
(a) Before irradiation														
Myeloblasts	—	—	—	—	0	103	0	—	—	—	—	0	69	0
Myelocytes immature	1	1	—	—	2	1 063	1 8	2	3	—	—	5	1 175	4
Myelocytes half mature	2	5	1	—	8	1 893	4 2	1	14	2	1	18	2 346	7 7
Myelocytes mature and meta	1	6	3	—	10	6 796	1 4	2	13	1	1	17	3 699	4 6
Mature granulocytes	—	—	—	—	0	11 104	0	—	—	—	—	0	10 035	0
Total	4	12	4	0	20	20 959		5	30	3	2	40	17 374	
Cells per 100 mitoses	20	60	20	0	100	104 795		17 5	75	7 5	5	100	43 310	
Mitotic index							0 95							2 3
(b) After irradiation														
Myeloblasts	—	—	—	—	0	0	0	—	—	—	—	0	35	0
Myelocytes immature	—	—	—	—	0	263	0	—	7	2	—	9	973	9 7
Myelocytes half mature	1	1	—	—	2	738	2 7	4	8	4	—	16	1 524	10 5
Myelocytes mature and meta	—	—	—	—	0	1 212	0	3	9	3	—	15	4 076	3 7
Mature granulocytes	—	—	—	—	0	18 746	0	—	—	—	—	0	5 096	0
Total	1	1	0	0	2	20 959		7	24	9	0	40	11 604	
Cells per 100 mitoses	50	50	0	0	100	1 047 950		17 5	60	22 5	0	100	29 010	
Mitotic index							0 09							3 4

examined before X ray treatment and 4 months after it, the total doses given over the splenic area being 800 r The total number of leucocytes corresponding to 20 mitoses was counted on both occasions (Table 25 and Fig 46 c)

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It is striking that the sternal marrow and also the remainder of the bone marrow except that in the area of irradiation *still show typical leukæmic changes with excess of immature forms and many mitoses even after a complete course of radio therapy in fact the typical changes may become more marked* A comparison of the blood picture with the myelogram shows that the increase in the number of mitoses in the marrow cannot be attributed to diminished admixture of granulocytes from the blood If such increase of mitoses in the bone marrow after irradiation of the spleen can be confirmed in other cases of chronic myelocytic leucosis we should have a reasonable explanation of the decrease of hormonal inhibition of the marrow which follows diminution in size of the spleen The only observation which we are aware is that of Stephens who observed 1 case in which 500 r were given over the thorax and spleen 5 months earlier He found that in the sternal marrow immature myeloid cells had fallen from 52 7 to 24 3 per cent Biopsy from the sternum before irradiation of the thorax showed increased hyperplasia of the myeloid tissue whereas 5 months later the marrow was poor in cells and fibrotic This case is not directly comparable with ours because the sternal marrow as well as the

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	Prophase	Monaster	Diaster	Dispireme	Total cells in mitoses	Number of cells per 1 mitoses	*Mitoses per 1 000*	Prophase	Monaster	Diaster	Dispireme	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000
(a) Before irradiation														
Myeloblasts	—	—	—	—	0	103	0	—	—	—	—	0	69	0
Myelocytes immature	1	1	—	—	2	1 063	1 8	2	3	—	—	5	1 175	4
Myelocytes half mature	2	5	1	—	8	1 893	4 2	1	14	2	1	18	2 346	7 7
Myelocytes mature and meta	1	6	3	—	10	6 796	1 4	2	13	1	1	17	3 699	4 6
Mature granulocytes	—	—	—	—	0	11 104	0	—	—	—	—	0	10 035	0
Total	4	12	4	0	0	20 959		5	30	3	2	40	17 324	
Cells per 100 mitoses	0	60	0	0	100	104 795		12 5	75	7 5	5	100	43 310	
Mitotic index							0 95							2 3
(b) After irradiation														
Myeloblasts	—	—	—	—	0	0	0	—	—	—	—	0	35	0
Myelocytes immature	—	—	—	—	0	263	0	—	7	2	—	9	923	9 7
Myelocytes half mature	1	1	—	—	2	738	2 7	4	8	4	—	16	1 524	10 5
Myelocytes mature and meta	—	—	—	—	0	1 212	0	3	9	3	—	15	4 026	3 7
Mature granulocytes	—	—	—	—	0	18 746	0	—	—	—	—	0	5 096	0
Total	1	1	0	0	2	20 959		7	24	9	0	40	11 604	
Cells per 100 mitoses	50	50	0	0	100	1 047 950		17 5	60	22 5	0	100	29 010	
Mitotic index							0 09							3 4

inhibition as a result of arsenic Erythropoiesis in the spleen shows distinct increase as a result of arsenic and in our second case (Table 27) it increased by 5 times within 3 weeks

TABLE 27

Spleen and Marrow before and after Arsenic Treatment

		Case 6 (34 male)				Case 14 (5_ male)			
		Before arsenic		After 16 days arsenic (Fowler)		Before arsenic		After 24 days arseni (Fowler)	
		Sternum	Spleen	Sternum	Spleen	Sternum	Spleen	Sternum	Spleen
100%	*Macrophages*	—	*0 1*	—	+	—	*0 2*	*0 2*	0 2
	Plasmacyt retic cells	—	0 3	0 1	1 1	—	0 1	0 2	2 6
	Pulp cells	—	—	—	0 1	—	—	—	0 6
	Erythroblasts basophile	1 0	1 5	7 7	2 9	0 8	3 2	2 0	3 8
	Erythroblasts polychromatic	4 8	8 7	13 2	11 7	1 5	5 8	18 6	27 6
	Erythroblasts oxyphile	0 9	3 2	0 7	1 2	—	1 6	7 2	16 2
	Erythroblasts total	6 7	13 4	21 6	15 8	2 3	10 6	27 8	47 6
	Myeloblasts	1 0	0 5	0 3	0 2	5 9	1 7	2 0	0 2
	Myelocytes immature	10 4	1 3	2 7	1 0	7 6	6 8	2 4	0?
	Myelocytes half mature	32 2	3 9	11 3	2 6	15 6	13 4	5 2	?8
	Myelocytes mature	6 0	5 5	5 9	3 4	2 6	13 1	2 2	5 0
	Metamyelocytes	8 0	12 2	7 1	3 9	6 2	9 3	5 4	3 6
	Total myelocytes	56 6	2_ 9	27 0	10 9	32 0	44 3	15 2	11 6
	Neutrophiles staff	*23 0*	*25 1*	*30 2*	*12 1*	*41 6*	*18 7*	*30 4*	*10?*
	Neutrophiles segmented	6 8	12 0	12 5	14 9	7 4	10 3	9 2	5 4
	Eosinophiles mature	3 0	2 2	4 8	6 3	3 9	2 2	8 4	5?
	Basophiles mature	1 2	3 0	1 2	4 7	5 1	5 0	4 2	0 8
	Monocytes	0 6	0 8	0 9	2 4	0 4	0 6	—	—
	Total lymphocytes	1 0	19 7	1 4	31 5	1 4	8 0	2 4	15 6
	Megakaryocytes	0 1	+	+	—	+	—	+	—
Hæmogram									
	Total leucocytes	81 200		11 500		179 000			
100	Myeloblasts	—		—		5			
	Myelocytes immature	4⅓		—		2			
	Myelocytes half mature	2⅓		1		10			
	Myelocytes mature	5		—		2½			
	Metamyelocytes	4		1½		5			
	Total myelocytes	16		2½		19½			
	Neutrophiles staff	41		_0		44			
	Neutrophiles segmented	31⅓		48½		13			
	Eosinophiles (myelos and mature)	1⅓		7½		3½			
	Basophiles	3⅓		3½		7½			
	Monocytes	2⅓		6½		2½			
	Lymphocytes	4		11½		5			

In the sternal marrow we were able to confirm the observations of Stephens *that the changes are similar to those in the spleen* although they are more striking on account of the initially more immature picture Thus in the first case the number of myelocytes fell to half while the mature granulocytes increased by a third and

erythroblasts to treble the initial number Case 14 (Table 27) showed a smaller decrease of immature granulocytes to one third of their initial number with almost complete disappearance of myelo blasts and immature myelocytes Within 3 weeks the erythroblasts had increased to eleven times their initial number

In Case 8 the splenogram which is shown in Table 22 cannot be amplified by myelograms because we obtained no good films This patient had been taking small doses of arsenic (8–12 drops of Fowler s solution twice a day) for 8 years and was continuously fit for work Initially the leucocyte count was in the region of 195 000 but for a number of years it remained between 8 000 and 12 000 with only 1 or 2 per cent of immature cells in the blood films Even the shortest interruption of the arsenic was immediately followed by a rise of granulocytes After 5 years continuous treatment with arsenic the splenogram showed a decrease of myelocytes to one quarter of its former value while immature forms were also greatly reduced (Table 22 Case 8) the erythroblasts having quadrupled themselves and the lymphocytes having doubled themselves

Thus treatment with arsenic results in a distinct percentage decrease of granulocytes particularly of the immature myelocytes both in the spleen and the marrow while there is a relative increase of lympho cytes in the former and erythroblasts in the marrow and sometimes in the spleen (Fig 46 *d*)

Effects on mitoses The far reaching therapeutic effect of arsenic on the leukæmic process is certainly not fully explained by the marrow although there is definite decrease of granulocytes in the spleen and marrow *According to our observations* (*Moeschlin* (19)) *arsenic is still the most powerful therapeutic agent in chronic*

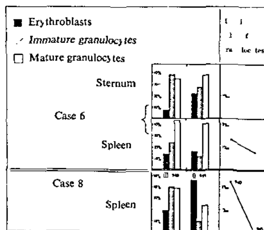


FIG 46 *d* Sternal and spleen films before and after arsenic treatment (For explanation see text)

myelocytic leucoses and is to be preferred to X rays or urethane for continuous treatment because it has also a beneficial effect on the expectation of life as Forkner has also stressed This treatment does not however have any effect on the terminal paramyeloblastic phases of chronic myelocytic leukæmias and, indeed, the patient just mentioned died of such an attack after 9 years of treatment

We have investigated the effect of arsenic on the number of mitoses in both the above cases because in view of Dustin s work it seems reasonable to assume that arsenic in the leukæmias acts as a mitotic poison Our results are shown in Table 28

The result of these investigations clearly shows that arsenic in therapeutic doses produces a well marked decrease in the number of mitoses in granulocytic tissues After 2 weeks treatment with arsenic the number of mitoses in the spleen fell to approximately one half and in the marrow to approximately one third of the values found before treatment commenced (Fig 46 *d*)

Such inhibition of mitosis persists during continuous treatment with doses of arsenic that are just sufficient to maintain the leucocytes at numbers slightly above normal The mitoses fall to very low values as is shown in Case 8 —

Before arsenic treatment	*After 5 years continuous arsenic treatment*
40 mitoses in 15 580 granulocytes	No mitoses in 15 580

In this case therefore the number of mitoses has decreased by at least 40 times as a result of persistent treatment with arsenic

The therapeutic effect of arsenic in the leukæmias therefore depends mainly upon the toxic inhibition of mitosis Further in analogy with the observations of Rohr on the effect of arsenic on erythropoiesis in pernicious anæmia there is possibly a beneficial effect on cellular maturation Certainly one of the greatest advantages of arsenic is the fact that it can be administered in therapeutic doses for years on end without damaging the formation of red corpuscles (Table 22 Case 8)

During arsenic treatment of myeloid leukæmias there is a shift to the left of the mitotic curve due to an increase in the number of prophases viz in the spleen from 5 per cent to 20 per cent and in the sternal marrow from 13 per cent to 25 per cent Fieschi asserts that this increase in the number of prophases depends upon the stimulation of karyokineses This is certainly not true of the mitotic curve of chronic leukæmia treated with arsenic because almost certainly there is a toxic delay in the course of prophase It is not possible to obtain any definite information as to the duration of mitosis by simple enumeration of the various stages and the

TABLE 28

Number and Distribution of Mitoses in Spleen and Sternal Marrow Before and After Treatment with Arsenic

Case 6 (*male aged 34*)

	Spleen							Sternum						
	Prophase	Monaster	Diaster	Dispireme	Total cell in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000 immature cell	Prophase	Monaster	Diaster	Dispireme	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000 immature cells
(a) Before arsenic														
Myeloblasts	—	—	—	—	0	227	0	—	—	—	—	0	146	0
Myelocytes immature	—	1	—	—	1	596	1 6	2	7	4	1	14	1 524	9 1
Myelocytes half mature	2	12	13	—	27	1 776	15 2	3	19	9	1	3	4 720	6 7
Myelocytes mature and meta	—	9	3	—	12	8 042	1 4	3	10	1	—	14	2 052	6 8
Mature granulocytes	—	—	—	—	*0*	*19 582*	*0*	—	—	—	—	*0*	*5 074*	*0*
Total	2	22	16	0	40	30 217		8	36	14	2	60	13 516	
Cells per 100 mitoses	5	55	40	0	100	75 542		13	60	23	3	100	22 5 6	
Mitotic index							1 32							4 43
(b) After arsenic														
Myeloblasts	—	—	—	—	0	101	0	—	—	—	—	0	55	0
Myelocytes immature	1	1	1	—	3	509	5 8	1	5	4	2	12	495	24
Myelocytes half mature	2	1	4	—	7	1 373	5 2	6	6	4	—	16	2 073	7 7
Myelocytes mature and meta	1	7	2	—	10	3 716	2 6	3	7	2	—	12	2 384	5 0
Mature granulocytes	—	—	—	—	0	20 570	0	—	—	—	—	0	9 101	0
Total	4	9	7	0	20	26 219		10	18	10	2	40	14 108	
Cells per 100 mitoses	0	45	35	0	100	131 095		25	45	25	5	100	35 270	
Mitotic index							0 76							2 83

conclusions of Fieschi and Kienle, who attribute the gradual shift of the mitotic curve to acceleration or retardation of the various stages of cell division, are purely hypothetical

THE EFFECTS OF URETHANE ON THE SPLEEN AND MARROW PICTURES IN THE LEUCOSES

In view of the observations of Haddow Sexton and Patterson on inhibition of tumour growth and its value in the treatment of chronic leucoses it seems useful to discuss the haematological changes that occur in the spleen and marrow after urethane

A brief description of the effects of urethane on the peripheral blood will be given as an introduction

Moeschlin (18) and Meili have shown that the usual therapeutic dose of urethane (3 g daily) causes a fall of lymphocytes in most normal people indeed, a genuine alymphocytosis may develop The granulocytes fall slightly after a preliminary rise, but in specially susceptible persons and in the presence of previous marrow damage, severe granulocytopenia may develop As a rule neither erythropoiesis nor thrombocytopoiesis is affected but, again, in susceptible persons, there may be decrease As Table 29a shows, there is no diminution of mitoses even after 4 weeks on urethane

TABLE 29a

Mitoses (Mitotic Index) before and after Urethane (Healthy Subjects)

	Case number	Before urethane	After urethane
Erythroblasts	1	14‰	32nd day 17 [illegible]
	2	1[illegible] /	13th day 1[illegible] 2[illegible]th day 14 /
	3	14 /	14th day 1[illegible] /
Granulocytes	1	1 3%	32nd day 0 [illegible] /
	2	0 9%	13th day 0 [illegible] / [illegible]th day 1 2 /
	3	1 3 /	14th day 0 8 /

A functional agranulocytosis without granulocytopenia can be produced by urethane in the normal individual as Sandkuhler (1949) has demonstrated The greatly lowered resistance against infections of cases undergoing urethane treatment can be explained by the fact that in healthy persons the application of daily 2 gr urethane lowers the phagocytic index from 80 to 50 per cent

In animal experiments (Moeschlin (20) and Naef) there was a distinct susceptibility of lymphatic tissue granulocytes and red

TABLE 29b

Myelogram and Splenogram Chronic Lymphatic Leukæmia before and after Urethane

	A A aged 33 male Myelogram			Splenogram	D L aged 59 female Myelogram			Splenogram	
	1st day	27th day	113th day	113th day	1st day	7th day	93rd day	1st day	93rd day
Erythroblasts	3 2	2 2	29 0	3 4	4 6	4 2	3 0	—	0 ∠
100 { Immature granulocytes	5 0	1 2	16 8	3 0	1 ?	0 6	1 0	0 1	0 1
Mature granulocytes	5 4	4 0	7 4	5 4	1 0	3 4	2 ?	1 6	6 4
Lymphocyte	89 6	94 8	75 8	87 5	97 8	96 0	96 8	98 2	93 3
Hæmogram									
Total leucocytes	19 000	5 000	5 600		81 00	46 350	17 ∠00	81 200	17 ?00
Immature granulocytes	5	—	3		—	—	—	—	—
Mature granulocytes	28	46	39		6 5	7	32 5	6 5	32 5
Lymphocytes	67	54	58		93 5	92 5	66 5	93 5	66 5
Thrombocytes	8 000	63 000			66 000	16 000		66 000	

cells being affected later The only exception was the cat, in which we found that the granulocytic system was as sensitive as that of patients with chronic myelocytic leucosis *Quite moderate doses of* urethane can produce fatal panmyelophthisis in cats (Moeschlin (20 26) and Bodmer) Clearly, then there is great variability in the susceptibility of different blood cells and also of different animals

(a) CHRONIC LYMPHATIC LEUCOSES

The therapeutic effect of urethane is usually less than in chronic myelocytic leucoses (for literature see Moeschlin (19, 21)) A slight reduction of lymphocytes is found in spleen and marrow punctures (Table 29b)

The great reduction of lymphocytes that may occur after a treatment of 1 month or longer in the peripheral blood before there is any or only little change in the cellular composition of the spleen and marrow shows that the main action of urethane is on cell proliferation as well as in reducing the amount of lymphatic tissue

Unfortunately *in the 1st and 3rd cases no spleen puncture was* carried out before treatment (because of an hæmorrhagic diathesis) but even so there was a demonstrable increase of extramedullary blood formation in the spleen (up to 6 per cent), presumably as a result of depression of lymphocytopoiesis

In fact after urethane the lymphocytes in spleen puncture may fall to 85–89 per cent unlike untreated cases in which they are usually above 95 per cent No determination of the mitotic index was possible because of the rarity of cell division in lymphocytes (see p 40)

We would stress the inadvisability of giving urethane in any case of aleukæmic lymphatic leukæmia in which there is leucopenia because there is usually very extensive infiltration of the marrow with lymphocytes In one such case urethane caused complete failure of the already inadequate marrow

(b) CHRONIC MYELOCYTIC LEUCOSIS

As in chronic lymphatic leukæmia urethane seems to have a slightly stimulating effect before its depressant one manifests itself as is shown by a slight increase of cells in the peripheral blood during the first few days of treatment (Moeschlin (17) Storti (3)) It seems probable therefore that the action of urethane is on cell division Within 7–14 days varying with the dose and the individual the granulocytes begin to fall (in lymphatic leucoses the decrease may commence much later *e g* 3–4 weeks)

Is this decrease brought about by destruction of the leukæmic cells ? If this were so the blood uric acid and the excretion of uric acid would arise as it does after radio therapy but we have not found this in any of our urethane cases Clearly, then the drug

TABLE 29c

Mitotic Index in Spleen and marrow Punctures in Chronic Myeloid Leukæmia before and after Urethane Treatment

	Case 1 (aged ? female)				Case 2 (aged 66 male)				Case 3 (aged 3 male)			
	Mitotic index per 1 000				Mitotic index per 1 000				Mitotic index per 1 000			
	Spleen		Marrow		Spleen		Marrow		Spleen		Marrow	
	1st day	35th day	1st day	35th day	1st day	38th day	1st day	38th day	1st day	32nd day	1st day	32nd day
Erythroblasts	8 9 /	0 /			6 2 /	5 9 /	1 /	14 5 /	11 ? /	8 5 /	1 7 ‰	16 3 ‰
Myeloblasts, Immature myelocytes, Half mature myelocytes, Mature myelocytes, Mature granulocytes	4 /	1 4 /			3 15 ‰	? 9 /	4 ‰	6 03 ‰	0 4 /	0 5 /	0 19 ‰	6 2 ‰

must act by inhibiting the formation of leukæmic cells Then again there are no distinctive structural changes suggestive of cell destruction (Storti (3) Moeschlin (17)), although the cytoplasm of some of the younger cells may be vacuolated This change also occurs in experimental animals (Moeschlin (20) Tischendorf) but is much less striking than the other phenomena Bock has also described clumping of the peroxidase granules which may lie at the edge of the cell Storti tells me that he also has observed this

Changes in Spleen Puncture Table 29c shows the findings in 3 cases in each of which 50 000 cells were examined in spleen and marrow films in order to enumerate the mitoses (I am deeply grateful to the Misses Ernst and Obrecht for this time consuming labour)

In the first case after 35 days on urethane the blood picture had become much more normal but apart from a distinct increase of erythropoiesis the proportions of granulocytes showed little change Apparently there had been considerable retrogression of leucopoietic marrow and increase of erythropoietic tissue although the number of immature white cells relative to the mature forms showed little change only the segmented neutrophiles had increased In this case the spleen only became 3 cm smaller in 35 days

How can the improvement in the blood picture be explained (the leucocytes fell from 194,000 to 25 000)? It is not really conceivable that the very moderate retrogression of the leucopoietic tissue in the spleen and marrow could be the cause of so great a metamorphosis there must have been the additional factor of inhibition of cell division The view is confirmed by our observations on mitotic activity Table 29c shows the number of mitoses per 50 000 cells Fig 47 *a* makes clear that the granulocyte mitoses in the spleen (after 35 days on urethane) fell to a quarter of the initial number while those of the erythroblasts were doubled

In the above case the improvement following urethane was due to a selective inhibition of granulocyte proliferation In the other 2 cases in which the number of mitoses was counted (Table 29c) there was a similar but less intense fall of the mitotic index in spleen films Storti (3) and Mauri as well as Schulze and Bock have confirmed our findings

Sternal Puncture As a rule there is a distinct rise in the mitotic index of the erythropoietic cells but rather surprisingly little change in that of the granulocytes indeed in Case 3 there was increase in the number of granulocyte mitoses Until further investigations have been carried out there can be no explanation of this peculiar difference between the conditions in the spleen and marrow The condition is however comparable with that following X irradiation of the spleen in which diminution in the size of the organ perhaps decreases the intensity of the splenogenic inhibition of the marrow

with a consequent rise of the mitotic index (p 144) The failure of urethane as an inhibitor of the marrow only is the more surprising because arsenic affects spleen and marrow equally

We have already shown that urethane has practically no inhibitory effect on myelopoiesis in the normal subject and it may be that the different response of the spleen and marrow in chronic leukæmias is due to a different mode of granulocytopoiesis in the two organs Thus it might be that only the spleen has undergone a

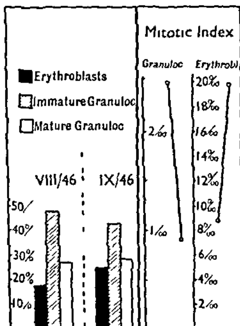


FIG 47 a *Effect of urethane on mitoses in the spleen in chronic myeloid leukæmia* After 35 days treatment with urethane (99 g) there is an increase of erythroblasts but the proportion of myeloid leucocytes shows little change. There is however decrease of the mitotic index of the granulocytes to one third of their original number *i.e* to the number of mitoses per 1 000 immature cells. *The mitotic index of the erythroblasts has risen to double the original value The main effect of urethane is thus a selective inhibition of mitotic activity in the abnormal leukæmic white cells*

leukæmic change and is therefore more sensitive to urethane The hypothesis is attractive but lacks evidence Thus splenectomy usually has a bad effect on chronic myelocytic leukæmia (Forkner Gasser *et al*) while as we have already pointed out the granulocytes of the marrow show also structural leukæmic abnormalities Clearly then this series of cells is atypical in both organs at least in the advanced stages of the disease Then again we have seen a terminal paramyeloblastic exacerbation commence in the marrow and not in the spleen (Case 2 p 160) We are therefore forced to accept the view that the apparent paradox is perhaps the result of

decreased inhibition of the marrow, as a result of decrease in the size of the spleen

Care is needed in the interpretation of mitotic indices Thus in tissue culture, Bucher observed differences in mitotic activity due to varying concentrations and duration of action of the chemical substances he tested It is therefore possible although not probable, that the explanation is that there are different concentrations of urethane in the spleen and marrow It is also possible that the marrow cells but not those of the spleen are regulated by nervous activity The field thus opened for hæmatological research is both extensive and important

We have purposely devoted space to Case I because the failure of the marrow and spleen cells to show signs of increased maturity demonstrates that the striking therapeutic effect of urethane cannot be explained by simple depression of the immature cells Admittedly in our other cases there was extensive metamorphosis of the cellular composition of the spleen and marrow, inasmuch as the immature cells decreased and there was a relative increase of more mature forms This might occur within 2 or 3 weeks but usually within 3 6 weeks This change is well shown in Table 29d (Case 3) Here the granulocytes in the spleen fell from 58 per cent to the very low figure of 0 7 per cent within 4 weeks while the lymphocytes rose from 12 per cent to become the predominant cells (67 per cent) At the same time the immature white cells fell to a quarter of their initial value and erythropoiesis became more active than normal In such cases the reversion to normal of the blood picture can be explained, even in the absence of any special interference with mitosis There is however inhibition of cell proliferation which affects the younger cells more than the less sensitive mitoses of the more differentiated cells (mature myelocytes) *At present such a selective action of urethane on the young leukæmic cells in the sense of inhibition of the cell proliferation seems to be the best explanation of the effects of urethane* Indeed as has been shown in animal experiments such damage of immature cells may even lead as far as to produce neoplastic degeneration (Henshaw Nettleship Guyer Jaffe Moeschlin (20 21) and Naef)

(c) STRUCTURAL CHANGES IN MITOSES

Not only does urethane cause a decrease in the number of mitoses but abnormal forms occur For instance in spleen films we have observed swollen chromosomes adhesion of pairs of chromosomes and displaced ones Bock Schulze and Storti have reported similar appearances in the marrow while Bastrup Madsen has seen them in tissue cultures of fibroblasts

Moeschlin (26) and Bodmer found identical structural changes in cats in which panmyelophthisis had been caused by urethane

TABLE 29d

Myclograms and Splenograms in Chronic Myeloid Leucoses (before and after Treatment with Urethane)

	Cas 1 R H (aged ? female)				Case K E (aged 64 male)				Case 3 P E (aged 3 male)			
	Sternum		Spl en		Ste num		Sple n		Sternum		Spleen	
	1st d y	35th d y	1st day	35th day	1st day	39th day	1st d y	39th day	1st day	3 nd day	1st day	3 nd day
Erythroblasts	5 8	37 0	2 0	32 7	6	19 0	7 1	4 4	3 3	59 4	10 0	17 6
100 Myeloblasts	4 4	1 0	4 1	—	1 0	0 8	—	—	5 2	1 9	11 7	—
Myelocyte total	44	47 6	54 3	57 8	51 0	41 4	36 2	16 4	36 9	9 4	46 0	0 7
My l cyte n n ature	28 2	9 8	30 1	40 4	37 0	35 6	22 0	11 3	?7 3	7 2	19 0	—
Myelocyt s mat e	6 0	8 6	9 6	3 2	14 0	5 8	14	5 1	9 6	2	27 0	0 7
Neut oph l staff	32 0 } 43	25 8 } 47 4	2 0 } 29 3	16 9 } 36 9	6 } 37 6	29 4 } 53 6	17 4 } 32 4	8 8 } 44 4	3 7 } 29 1	8 0 } 15	3 7 } 1 4	? 3 } 10 6
Neut oph les segn ted	11 2	21 6	7 3	20 0	11 4	4 ?	15 0	35 6	5 4	7 2	8 7	8 3
Eos noph les	1 0	0 4	1 0	—	5 6	1 ?	0 9	2 7	14 5	9 5	7 6	5 3
Basophile	4 4	0 2	3 7	0 9	3 8	0	9 6	11 5	8 1	3 6	0 3	—
Mono ytes	—	—	—	0 1	—	—	—	1 0	—	—	—	—
Lymphocyt s	8	3 4	7 6	4 2	1 0	1 0	0 6	4	2 5	0 9	1 3	63 6
Hen g m												
Leu ocytes	193 600	25 400	193 600	25 400	74 100	15 900	74 100	15 900	111 400	9 900	111 400	9 900
Myelobl st ( )	4	2 5	4	2 5	—	—	—	—	9	—	9	—
Myelocytes total ( )	50	32	50	32	5½	—	5½	—	16	2	16	2
Myelo ytes n mat re ( )	6 5	17	6 5	12	5¼	—	5¼	—	11		11	
Myelocytes m t e ( )	10 5	1 5	10 5	12 5	¼	—	¼	—	5	—	5	—
N utrophiles staff ( )	33 } 39	1 5 } 68 5	33 } 39	1 5 } 68 5	4¾ } 75¼	11 5 } 66 5	24¾ } 75¼	11 5 } 66 5	14 } 7	4 } 57	14 } 77	4 } 57
Ne trophiles segmented ( )	6	47	6	47	50½	55	50½	55	13	53	13	53
Eos ophil s ( )	0 5	0 5	0 5	0 5	1½	4 5	1½	4 5	21	1	21	1
B oph les ( )	3	1 5	3	1 5	11¼	0 5	11¼	0 5	23 5		3 5	—
Mo o ytes ( )	—	1	—	1	1¾	4 0	1¾	4 0	0 5	—	0 5	—
Lympho ytes ( )	3 5	3 5	3 5	3 5	4¾	24 5	4¾	4 5	4 0	7	4 0	7
Thrombocytes	153 000	107 500	153 000	107 500	540 000	—	540 000	—	746 400	—	746 400	—

decreased inhibition of the marrow, as a result of decrease in the size of the spleen

Care is needed in the interpretation of mitotic indices Thus in tissue culture Bucher observed differences in mitotic activity due to varying concentrations and duration of action of the chemical substances he tested It is therefore possible although not probable that the explanation is that there are different concentrations of urethane in the spleen and marrow It is also possible that the marrow cells but not those of the spleen, are regulated by nervous activity The field thus opened for hæmatological research is both extensive and important

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TABLE 29e

Myelograms and Splenograms during Continuous Treatment of Chronic Myeloid Leucoses with Urethane

	Case 1 (B H aged 55 male)								Case 2 (D L aged 37 male)	
	Sternum				Spleen				Sternum	Spleen
	1st day	276th day	550th day	653rd day	1st day	276th day	550th day	653rd day	150th day	150th day
Erythroblasts	27 2	19 4	21 6	13 0	47 6	17 6	14 7	6 2	6 2	0 1
Myeloblasts	0 6	1 6	5 6	27 6	0 2	4 2	—	6 2	46 3	0 1
Myelocytes total	12 8	35 2	28 0	37 6	11 6	20 0	16 9	46 6	11 2	21 6
Myelocytes immature	6 9	26 8	19 0	28 4	3 0	5 6	5 6	18 6	9 7	4 4
Myelocytes mature	5 9	8 4	9 0	4 4	8 6	14 4	11 3	28 0	1 5	17 2
Neutrophiles staff	23 9	25 6	27 6	3 8	10 2	11 8	3 7	5 4	3 7	4 6
Neutrophiles segmented	7 2	5 4	5 4	1 0	5 4	10 4	32 0	6 2	3 9	63 9
Eosinophiles	6 7	6 0	2 6	—	5 2	1 0	2 0	—	4 7	1 6
Basophiles	3 3	5 6	7 2	23 4	0 8	7 8	20 0	3 2	7 8	7 1
Monocytes	—	—	—	—	—	—	0 7	—	15 7	0 2
Lymphocytes	1 9	0 8	0 4	0 6	15 6	26 4	9 7	25 0	—	0 6
Plasma cells	—	—	—	—	—	—	—	0 2	—	—
Hæmogram										
Leucocytes		11 800	33 900	1 700		11 800	33 900	1 200	18 400	
Myeloblasts		—	8½	1		—	8½	1	—	
Myelocytes total		10½	30	28		10½	30	28	5	
Myelocytes immature		9½	22	16		9½	27	26	3	
Myelocytes mature		1	8	2		1	8	2	2	
Neutrophiles staff		14	19	13		14	19	13	6	
Neutrophiles segmented		51½	29	29		51½	29	29	67½	
Eosinophiles		1½	2½	—		1½	2½	—	1½	
Basophiles		13	10	14		13	10	14	18	
Monocytes		4½	—	5		4½	—	5	[illegible]	
Lymphocytes		5	1	10		5	1	10	1½	
Plasma cells		—	—	1		—	—	1	—	
Thrombocytes										

Obviously then urethane has an effect direct or indirect, on the course of cell division a matter we shall return to at the end of the present section

Enumeration of the different phases of mitosis revealed only a

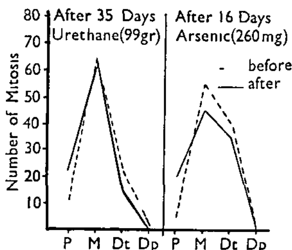


FIG 47 *b* *Distribution of the various stages of mitosis in chronic myeloid leukæmia before and after treatment (spleen puncture)* P = prophases M = monaster Dt = diaster Dp = Dispireme No striking change in the mitotic curve can be detected but there is slight increase in the number of prophases This is however within the limits of experimental error

slight increase of prophases (Fig 47 *b*) so that it is clear that urethane does not act only on one stage of cell division but on the whole karyokinetic process (Moeschlin (17) Schulze Storti (3))

(d) PROLONGED TREATMENT WITH URETHANE

Table 29e shows splenograms and myelograms from 2 cases of chronic myelocytic leucosis which were continuously treated with urethane

We could follow the first case for 3 years From 1945 until April 1946 the patient was treated with arsenic by mouth and was then given urethane At first 4 g were given daily but the dose was gradually reduced to between 0 5 and 1 5 g daily according to the state of the blood picture and during the whole period the patient was fit for work In 18 months the total amount of urethane was 800 g Every attempt to stop urethane was followed by rapid increase of leucocytes and deterioration of the general condition In the spring of 1948 there was a sudden fall of the white cells (to 7 000) and for the first time severe anæmia (56 per cent) In spite of stopping urethane and giving transfusions and penicillin the patient died with a severe panmyelopathy the granulocytes having fallen to 350 and the thrombocytes to 5 000 before death Puncture of the spleen and marrow excluded the possibility of a terminal paramyeloblastic exacerbation

The damage of which there were no premonitory signs was

enlargement of the spleen and lymph glands it is not always true There seems to be aggressive forms of paramyeloblastic leucosis discharging the cells directly from the marrow into the blood stream

(e) ACUTE LEUCOSIS

Paramyeloblastic leucoses may run a fairly chronic course even without treatment but even so there is some evidence that in some cases urethane has a depressant effect on the abnormal cells in the blood (Moeschlin (19)) We have never been able to observe a revival of myelopoiesis such as Schwarz Tieni and Heilmeyer described each in one special case indeed in spite of urethane all our cases ran the usual fatal course Once we had a case in which the spleen and sternal punctures showed almost total disappearance of abnormal cells from the blood spleen and marrow and a further oddity was that the acute leukæmia had developed in a case of congenital hæmolytic jaundice We have now seen this combination three times (Moeschlin (3))

Sch H aged 26 book keeper

One sister developed hæmolytic anæmia in 1933 The patient had a first attack of the same condition at the age of 12 (1933) and had had an enlarged spleen ever since

In March 1937 he was getting paler while the spleen was noticed to be increasing in size (Hb 30 per cent)

On admission to the Clinic the general condition was poor The spleen was enormous (31 × 21 cm) firm and tender The liver was enlarged (17 cm in the nipple line) and firmer than normal Hæmoglobin 20 per cent red corpuscles 1 18 million reticulocytes 7 8 per cent leucocytes 102 000—85 per cent oxydase positive micromyeloblasts containing azure granules and 4 per cent myelocytes Thrombocytes 38 000 Some spherocytosis Diminished osmotic resistance of red corpuscles 0 66–0 30 B S R 85 bilirubin 3 5 mg per cent serum iron 270 gamma prothrombin 30 per cent

Course The leucocytes fell to almost normal numbers after treatment with arsenic but the composition of the white-cell picture did not become less abnormal Repeated transfusions raised the hæmoglobin to 60 per cent The results of spleen and marrow punctures are shown in Table 29 *f*

In spite of continued administration of arsenic (8 drops t d s) there was sudden exacerbation at the end of 3 months the leucocytes having risen to 169 000 (all micromyeloblasts) When this relapse occurred urethane was started (4 g daily) and within 3 weeks the leucocytes had fallen to 1 500 Urethane was stopped after 87 g had been given but there was no reappearance of normal cells Spleen and marrow punctures now showed only reticulum cells and a few isolated micromyeloblasts (Table 29 *f*) Penicillin sulphonamides and repeated transfusions (34 in all) prevented the onset of intercurrent infection during this stage of secondary agranulocytosis (leucocytes between 100 and 300 for a month) Death from heart failure on June 19th 1947

Pathologically (Professor von Meyenburg) the marrow was reticular and devoid of cells indeed this condition was so extreme that the pathologist could not have made a diagnosis without the clinical history

certainly due to urethane but was not stopped by ceasing to give the drug *We should therefore, beware of continuous treatment with urethane, especially as Patterson Webster and others have recorded similar cases*

In our case spleen puncture which had shown considerable retrogression of granulocytopoiesis while arsenic was being given showed even further retrogression after 9 months on urethane while the lymphocytes rose to 48 per cent After a further 9 months (November 1947) there was a striking increase of basophiles (20 per cent) while shortly before death there was increase of immature white cells and great deficiency of neutrophiles and lymphocytes Post mortem puncture showed the presence of many immature cells while histological examination reveals the presence of many young granulocytes in the spleen and marrow without signs of maturation although there was no real myeloid aplasia Presumably therefore urethane had induced complete cessation of cell proliferation in both the spleen and the marrow

The second case treated for 5 months with urethane (Table 29e, No 2) is specially interesting because it is until now the only observation in which the terminal paramyeloblastic change did not start in the spleen but in the marrow as comparative counts clearly demonstrate Thus the splenogram showed few myeloblasts (0 1 per cent) almost all (3 5 per cent) being normal with 68 5 per cent of mature neutrophiles At the same time the sternal marrow contained 57 5 per cent of immature cells 46 3 per cent being paramyeloblasts and promyelocytes But as the hæmogram shows there were no paramyeloblasts in the blood at this stage they suddenly appeared 6 days later reaching 383 000 shortly before death Autopsy (Professor von Meyenburg) confirmed our finding of an almost completely paramyeloblastic marrow and a spleen typical of chronic myelocytic leucosis *i e* filled with strikingly mature cells

This case demonstrates that the terminal dedifferentiation may commence in the spleen or in the marrow As we have emphasised before granulocytopoiesis must be of leukæmic type in both organs not only in the spleen as some writers have suggested Also it is clear that such terminal paramyeloblastic exacerbations are identical with primarily acute leucoses In these cases also there is an initial infiltration of the marrow with paramyeloblasts before many such cells can be found in the circulation it is so to speak an acute aleukæmic paramyeloblastic leucosis The irruption of these cells into the blood is a secondary manifestation Obviously there is not necessarily much involvement of extramedullary foci in these cases although Rohr (2) assumed that this was always present His view is probably true of the chronic myeloid type because the cells do not grow in an aggressive manner whereas in the paramyeloblastic form as in this case and in similar forms without leukæmic

It is conceivable that treatment in an early stage *before* normal hæmopoiesis has been destroyed may result in the destruction of all leukæmic cells that means a cure e g, with some of the newer derivatives of aminopterin

(f) DISCUSSION AND SUMMARY

It has been clearly shown that there are considerable differences of susceptibility to urethane in different hæmatological cells and different animal species On the whole lymphocytes are the most susceptible cells in the normal organism *but the pathological granulocytes of chronic myeloid leukæmia possess a peculiar and specific sensitivity this is regarded as further evidence of their neoplastic character* Oddly enough the normal granulocytes of the cat are almost as sensitive as are the pathologically deranged neoplastic cells in human leukæmia

What can be the basis of the great differences of susceptibility ?

Todd Plentl Schoenheimer and Kelemen have suggested that urethane interferes in the synthesis of nucleoproteins and so indirectly with mitosis The differences in susceptibility would then be explained by differences in the composition of cytoplasm and nuclei and it would have to be assumed that the composition of pathological cells is different from that of normal stem cells Thus the action of urethane would be analogous to that of stilbamidine (Snapper (2)) and antimony (Rubinstein) on myeloma cells which directly interfere with the synthesis of nucleoproteins We are not however convinced by these arguments

Our observations have shown that urethane has a stimulating as well as an inhibitory effect on cellular proliferation We have already mentioned the preliminary phase of stimulation in the beginning of the treatment and also the rise of lymphocytes that precedes a fall in normal persons while the same is true of experimental animals including mice (Moeschlin (20)) In tissue cultures Bucher observed increased mitotic activity when urethane was first added We feel therefore that urethane must be regarded as having a direct or an indirect action on cell division and this idea is supported by the observation that urethane can induce tumour formation in animals (Henshaw Nettleship Claus Jaffe Moeschlin (20 21)) Further Vogt (1948) has been able to induce both recessive and dominant mutations in drosophilia with urethane Then also the changes that urethane induces in the structure of mitoses must not be forgotten

These three facts—stimulation before inhibition tumour formation and structural changes in mitosis—seem to suggest a direct or indirect action of urethane on cell proliferation i e on the latter end of division This is much like the view of Dustin Ludford and Moellendorf that urethane is a direct mitotic poison

The spleen was practically an empty reticular framework in which neither lymphocytes nor myeloid cells could be found

The lymphatic tissue of the glands spleen and tonsils showed advanced atrophy Spleen 1 8 kg liver 2 5 kg The only persistent sign of a pre existing leucosis was the presence of hæmorrhages

Before treatment with urethane the blood picture was aleukæmic but the spleen was infiltrated with 97 per cent of paramyeloblasts while as an indication of the presence of congenital hæmolytic anæmia, there were some macro erythroblasts and macrophages containing hæmosiderin The sternal marrow was also almost replaced by paramyeloblasts (88 per cent) After 87 g of urethane had been given the abnormal cells almost disappeared from the blood spleen and liver for about 3 weeks But the exact nature of the cells could not always be ascertained because of their close resemblance to small reticulum cells Histologically all that was left were a few reticulum cells lying in the almost empty meshes of those organs that had been packed with leukæmic elements This case is very similar to one described by Schwarz Tiени

TABLE 29f

Myelogram and Splenogram in Acute Myeloid Leucosis before and after Treatment with Urethane

	Sch H aged 26 male			
	Sternum		Spleen	
Erythroblasts	10 6	2 8	—	0 4
Paramyeloblasts	87 6	95 6	97 4	98 [illegible]
Myelocytes immature	—	—	—	0 2
Myelocytes mature	0 6	1 4	0 4	0 2
Neutrophiles staff	0 4	0 2	1 4	0 2
Neutrophiles segmented	—	—	0 4	0
Eosinophiles	0 6	—	0 2	0 6
Basophiles	0 2	—	—	—
Hæmogram				
Leucocytes	102 400	3 000	102 400	3 000
Paramyeloblasts	85½	70	85½	70
Myelocytes immature	3	—	3	—
Myelocytes mature	2½	—	2½	—
Neutrophiles staff	5½	16	5½	16
Neutrophiles segmented	3	10	3	10
Eosinophiles	1	1	1	1
Basophiles	—	1	—	1
Monocytes	½	2	½	2
Lymphocytes	—	—	—	—
Thrombocytes	38 000	—	38 000	—

These 2 cases show that urethane can occasionally suppress pro liferation of the abnormal cells completely although there is no revival of normal blood formation Then life can only be preserved for a time by chemotherapy and repeated transfusions

NITROGEN MUSTARD

As far as we know there are no reports of the effects of this substance on the cellular composition of spleen films Block (1948) has reported on the histology of material obtained by spleen puncture in a case of chronic myeloid leukæmia He found no changes 44–96 hours after an injection of 0 1 mg/kg —there was no clinical improvement On the other hand he found a distinct decrease of lymphocytes in the glands in a case of lymphatic leucosis

The clinical effects which closely resemble those of radiotherapy, have now often been described (*e g* Karnofsky) Aleksandrowicz *et al* found that of the hæmic elements the granulocytes lymphocytes and thrombocytes were the most sensitive while erythroblasts were much less so Reticulum cells increase in number and in phagocytic activity

TABLE 29g

K E aged 66 male

	Before treatment			Four weeks after treatment with nitrogen mustard		
	Hæmogram	Splenogram	Myelogram	Hæmogram	Splenogram	Myelogram
Erythrocytes	3 38 mill	—	—	[illegible] 87 mill	—	—
Leucocytes	78 000	—	—	30 400		—
Thrombocytes	51 000		—	42 000	—	—
Lymphoid retic cells	—	—	—	—	—	0 8
Erythroblasts	—	11 8	3 0	1 0	11 0	13 0
Myeloblasts and promyelocytes	1 0	3	4 8	0 5	1 2	3 4
Total immature granulocytes	13 0	56 0	33 6	10 5	3[illegible] 6	0 8
Neutrophiles taff	40 0	17 8	34 8	1 0	2[illegible] 4	34 4
Neutrophiles egmented	3[illegible] 5	10 2	[illegible]1 6	55 5	[illegible]5 0	27 2
Eosinophiles	5 5	2 0	[illegible] 2	0 5	2 0	1 0
Basophiles	6 0	0 6	3 4	10 5	1 6	[illegible] 2
Monocytes	1 0	—	—	0 5	—	0 2
Lymphocytes	1 0	1 6	1 4	1 5	2 4	0 4

We have investigated a man of 66 with chronic myeloid leukæmia who had 11 mg of nitrogen mustard after having been treated with arsenic for 4 years and later with urethane As he had become refractory to these methods we waited for 2 weeks and then tried nitrogen mustard Table 29g shows the blood pictures before and 4 weeks after the commencement of the treatment together with the myelograms and splenograms The general effect was a reduction of immature forms in both the marrow and the spleen being rather

In analogy with inhibition of respiratory ferments (Keilin and Hartree, 1939) and blockading of the sulphonamide effect on bacterias by urethane (Allgower) the effect of this substance may well be supposed to inhibit or destroy some enzyme system essential for cell proliferation If so, it would follow that the remarkable susceptibility of the stem cells of some groups was due to possession of specially urethane sensitive enzymatic processes

Investigations on these lines may throw light on the metabolic processes characteristic of neoplastic cells Quastel showed that urethane has a special action on tissue respiration while Bock found a distinct decrease in the utilisation of oxygen by tissue cultures after addition of urethane This is specially significant because Schulze has called attention to the fact that normal cellular respiration is pre requisite for normal cell division (Fisher and Stern, Ormsby and Fisher)

Arsenic also decreases the intra cellular respiration of some cells by combining with sulphydryl radicles (Welfare)

Brock Ducrey and Herken added 165 gamma/c c of urethane to tissue slices and to sea gull eggs—and found that *after preliminary increase of oxygen consumption* there was a distinct fall This is in conformity with our observations on the initially stimulating effect of urethane on h emopoietic cells

A difficulty in accepting the view that urethane acts by inhibition of enzyme systems is the lengthy persistence of its effects after its administration is stopped Thus in a case of Parkinsonism Moeschlin and Meili saw the first increase of granulocytes 18 days after cessation of urethane Even if the period of maturation of granulocytes (8 days) is subtracted (Moeschlin (12 15)) there are still 10 days during which granulocytopoiesis remains on a reduced level The same is true of the fatal cases of panmyelophthisis due to urethane

It seems unlikely that such persistence is due to individual variations in absorption excretion or detoxication of urethane Perhaps this long persistence is in relation with the complete destruction or neutralisation of some essential enzyme systems

The fact that urethane can induce tumour formation indicates the need for care in its use in man Certainly in this respect urethane resembles other tumour inducing agents such as arsenic and radium which act by altering the mitotic process—and presumably urethane also acts by a similar mechanism—not as a cytostatic agent (Heilmeyer (6)) as for instance stilbamidine on myelomas

It is of the utmost interest that arsenic X rays radium and urethane (and perhaps some œstrogens) can both inhibit and initiate neoplastic processes Whatever may be the differences between these agents they have in common the power of interfering with the complicated processes of cell division

The effects are similar to those of urethane viz reduction of the number of immature cells in the spleen the immature myelocytes and their precursors fell to a third of their previous values while there was a slight increase of erythropoiesis In the marrow the reduction of immature cells was a good deal less striking Six weeks after the treatment the mitotic index had risen presumably because a new generation of cells had started to proliferate Probably puncture at the end of treatment would show diminution of mitotic activity but this is likely to be transient Here as in the case of urethane it is interesting to find that the effect on the spleen is greater than that on the marrow No valid conclusions can be drawn until a number of other cases have been investigated by spleen and marrow punctures

3 PARAMYELOBLASTIC LEUCOSIS

We have already said that we have replaced the concept of acute leukæmia by that of paramyeloblastic leucosis because the disease may run a chronic course or even if it is apparently acute may have developed insidiously over quite a long time

By *paramyeloblasts* we mean the abnormal sometimes granular cells which gradually replace the granular leucocytes in the blood and marrow and which unlike the cells found in chronic myelocytic leucoses show only a very slight tendency to maturation never attaining the polymorphonuclear stage (Fig 47 *c*)

A chronic myelocytic leucosis may end as a paramyeloblastic leukæmia in which one proliferating cell type which has lost all power of further differentiation is overgrowing the other cells leading finally to death (Fig 47 *d*)

The *spleen* is not usually enlarged in the aleukæmic stage (Moeschlin and Rohr (3)) but is frequently palpable in sub leukæmic and leukæmic cases It is interesting to note that in such cases injection of 1 mg of adrenalin intramuscularly is followed by decrease in the size of the spleen and a great rise of paramyeloblasts in the blood e g from 70 000 to 700 000 in Hortling s case As regards the great tendency to bleeding spleen puncture has only been performed in those cases in which there was no undue inclination to hæmorrhage (Table 29i)

Our cases were of sub leukæmic type accompanied by definite splenomegaly In Cases 1 2 and 4 the infiltration of the spleen with paramyeloblasts was in a very early stage whereas in the third and last case almost all other normal spleen cells had been thrown back by the paramyeloblasts Mitoses were found in the paramyeloblasts in every case clearly showing that these cells had really been obtained from the spleen

Paramyeloblasts in both spleen and marrow films were distinctly less mature than those in the peripheral blood i e their nuclei and

more marked in the latter In the sternal marrow mature neutrophiles rose by one fifth whereas they were doubled in the spleen Erythropoiesis increased in the marrow after nitrogen mustard but less than after arsenic or urethane but this did not occur in the spleen

It was not possible to determine the effect on the mitotic index because the inhibition due to arsenic had not yet worn off (mitotic index 0 9 per 1 000) Three weeks after the last injection of nitrogen mustard the index was 2 2 per 1 000 Probably in cases that have not had previous treatment there would be a fall in the mitotic index

RADIO ACTIVE PHOSPHORUS

Since Lawrence introduced this isotope of phosphorus into the treatment of chronic leucoses there have been many publications on the subject Apparently the immediate results are excellent but relapse soon occurs Table 29h shows the effects of 24 millicuries on the condition of the spleen and marrow in a case that had been treated with urethane 18 months earlier, and with arsenic more recently

TABLE 29h

P E aged 31 male

	Before treatment			Six weeks after P 37 treatment		
	Hæmogram	Splenogram	Myelogram	Hæmogram	Splenogram	Myelogram
Erythrocytes	2 5 7mill			1 93 mill		
Leucocytes	35 400			17 000		
Thrombocytes	47_ 900					
Macrophages	—	½	—	—	—	—
Plasmacyt retic cells	—	½	0 4	—	—	0 2
Lymphoid retic cells	—	—	2 4	—	—	9 [illegible]
Pulp cells	—	—	—	—	1 3	—
Erythroblasts	4	2	1 8		9 4	8 0
Myeloblasts and promyelocytes	17	34½	20 0	11 5	12 7	16 7
Myelocytes immature	6½	10	6 4		8 0	2 4
Myelocytes mature	3	15½	7 4		25 3	2 2
Neutrophiles staff	8	9	14 8	7 0	7 7	9 0
Neutrophiles segmented	13½	10	12 4	10 0	4 3	7 2
Eosinophiles	12½	11	14 2	11 0	8 4	15 8
Basophiles	34½	2½	21 6	43 0	10 7	26 8
Monocytes	—	—	—	—	0 7	—
Lymphocytes	5	4½	3 2	10 5	11 3	3 0
Plasma cells	—		—	—	0 3	—

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P E aged 31 *male*

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	Hæmogram	Splenogram	Myelogram	Hæmogram	Splenogram	Myelogram
Erythrocytes	2 5 7mill			1 93 mill		
Leucocytes	35 400			17 000		
Thrombocytes	47 900			—		
Macrophages	—	½	—	—	—	—
Plasmacyt retic cells	—	¼	0 4	—	—	0
Lymphoid retic cells	—	—	2 4	—	—	9 2
Pulp cells	—	—	—		1 3	—
Erythroblasts	4	[illegible]	1 8	—	9 4	8 0
Myeloblasts and promyelocytes	17	34½	20 0	11 5	12 7	16 2
Myelocytes immature	6½	10	6 4		8 0	2 4
Myelocytes mature	3	15½	7 4		5 3	7
Neutrophiles staff	8	9	14 8	7 0	7 7	9 0
Neutrophiles segmented	13½	10	12 4	10 0	4 3	7 7
Eosinophiles	12½	11	14 2	11 0	8 4	15 8
Basophiles	14½	2½	21 6	43 0	10 7	6 8
Monocytes	—	—	—	—	0 7	—
Lymphocytes	5	4½	3	10 5	11 3	3 0
Plasma cells	—		—	—	0 3	—

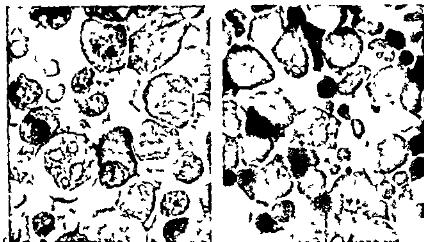


FIG. 47 Spleen films in paramyeloblastic leucoses

(c) Large partially lobulated paramyeloblasts in a case of so-called monocytic leukæmia

(d) Numerous vacuolated types together with erythroblasts and myelocytes indicating that in this case the acute leucosis has to be regarded as being the terminal paramyeloblastic stage of chronic myelocytic leucosis

The differences between the abnormal (neoplastic) cells and those of the normal myeloid series are particularly well seen in micro myeloblastic leucoses Thus in Case 5 we saw almost naked microparamyeloblastic nuclei (with a few Auer bodies) as well as rather large but normal myelocytes—and no transitional forms *We consider this co existence of a distinct pathological cell s ream and the normal (though scanty) myelopoietic row as further evidence of the neoplastic nature of the leukæmias*

Mitoses Many beautiful mitotic figures can often be found in paramyeloblasts but as these are abnormal (probably neoplastic) elements the mitoses differ considerably from those of normal myeloblasts and myelocytes

Isaacs (1930) was the first to describe the occurrence of haploid mitoses in leukæmic cells *i e* karyokineses in which there are 12 instead of 24 chromosomes in prophase or 24 instead of 48 in anaphase (diaster) Groat (1933) as well as Moeschlin and Rohr (1939) have confirmed this As such haploid mitoses have only been found in malignant tumours both carcinoma and sarcoma *we consider that their occurrence in the leukæmias is one of the strongest evidences of the neoplastic nature of these diseases* Certainly we are convinced that such abnormalities of cell division are not corre sponding to a hyperplasia or to Naegeli s irreversible dis turbance of correlation

TABLE 29ı

Paramyeloblastic Leukæmias

Case number	1	2	3	4	5
Age sex	74 ♀	51 ♂	23 ♀	57 ♂	26 ♂
Cell type	premyelocytes	monocytoid	immature myeloblastic	monocytoid	micromyeloblastic
Spleen (in cm)	12	13	[illegible]0		38
Hæmogram					
Hæmoglobin (in %)	69	31	69	66	60
R C (in millions)	—	—	2 872	3 0	2 66
Platelets	reduced	reduced	4 / = 11 000	27 / = 81 000	reduced
Leucocytes	2 [illegible]00	6 300	12 900	3 900	23 000
Paramyeloblasts (in %)	17½	87½	61	54½	94½
Paramyeloblasts absolute	385	5 512	7 869	2 126	21 735
Total myelocytes (in %)	1	—	3½	½	1
Neutrophiles staff	20	½	8	3	2½
Neutrophiles segmented	6	½	12½	9½	—
Eosinophiles	3	—	½	1½	1
Basophiles	4	—	—	½	1½
Monocytes	4	—	1	—	—
Lymphocytes	44	10½	13½	30½	—
Sternal marrow					
Paramyeloblasts ( )	40	—	99		95 6
Splenogram					
Macrophages	—	0 3	—	0 4	—
Plasmacyt retic cells	1 5	2 6	0 1	2 4	—
Pulp cells	0 1	—	—	1 0	—
Erythroblasts	0 1	0 2	1 2	4 8	0 4
Paramyeloblasts	10 7	[illegible]8 6	96 2	28 6	98 2
Paramyeloblasts in mitoses	+	0 2	0 [illegible]	0 2	0 2
Total Myelocytes	0 4	—	0 9	1 6	0 4
Neutrophiles staff	9 1	0 2	0 5	3 2	0 2
Neutrophiles segmented	8 2	0 1	0 1	2 0	0 2
Eosinophiles	1 2	—	0 1	2 8	0 6
Basophiles	1 5	—	—	0 6	—
Monocytes	0 8	0 1	0 [illegible]	—	—
Total lymphocytes	65 2	62 3	0 5	5[illegible] 6	—
Plasma cells	1 2	5 6	0 2	—	—

nucleoli were larger while the cytoplasm was narrower (Fig 47 *c d*)

Histological examination of embedded material from spleen puncture in Case 2 shows clearly that *in the early stages of infiltration the abnormal cells are in a focal arrangement reminiscent of sarcomatous metastasis* Fig 48 shows paler areas (↗) which correspond to collections of monocytoid paramyeloblasts while the remainder of the spleen preserves its normal structure

In all 5 cases a few erythroblasts and myelocytes were found but the latter did not show any signs of leukæmic abnormality and there were no transitional forms between them and the paramyeloblasts For this reason we regard their presence as being due to a compensatory myeloid metaplasia following the decrease in size of the medullary spaces by proliferation of the abnormal cells (p 61)

there are any pathognomonic changes in the blood or spleen If *however the marrow is not characteristic of myeloid leukæmia* although some cells resembling paramyeloblasts are found in the blood the possibility of a reticulosis or lymphosarcoma must be *considered (p 176) Then spleen or gland puncture may confirm* the diagnosis

(For an account of the possible effects of urethane in para *myeloblastic leukæmia see the previous chapter*)

4 POLYCYTHÆMIA VERA

We have included this disease among the hæmoblastoses because modern opinion basing itself on the analogy of the leukæmias tends to regard it as being a non malignant neoplastic proliferation of the erythroblastic system (Weber Minot and Buckman Nolli and Benaroio Rohr) in which the parent cells like those of chronic myelocytic leucosis have retained a good deal of their power of maturation Admittedly Heilmeyer and Apitz do not agree with this view

The spleen is usually enlarged and Heilmeyer attributes this to several factors viz increased reservoir function increased hæmolytic activity and least of all to the presence of ectopic foci of hæmopoiesis

We have no personal experience of spleen puncture in this condition Weil (1936) did not find any striking increase of erythro blasts an observation that supports Heilmeyer s view Weil cites the following as a typical splenogram —

Erythroblasts 5 per cent premyelocytes 2 per cent neutro philes 46 per cent monocytes 3 per cent and lymphocytes 44 per cent

Dedichen (1941) recorded the case of a man of 44 with hæmoglobin 109 *per cent red corpuscles 6 8 millions and 24 000 leucocytes* of which 5 per cent were myelocytes In spleen films there were many myelo cytes a few megakaryocytes and many erythroblasts The photographs that accompany his report as well as the myelogram which revealed a hyperplastic but not specially erythroblastic marrow make it clear that this was in fact a case of chronic myelocytic leukæmia It is recognised that polycythæmia may occur in the early phases of this malady (Naegeli (1) Schwarz 1939 and Case 13 on p 174)

In another case of a woman of 44 with typical polycythæmia Dedichen s spleen puncture revealed only lymphocytes and occa sional erythroblasts but neither myelocytes nor megakaryocytes

There is no doubt that transitions between polycythæmia vera and chronic myelocytic leucosis do occur (Naegeli Rachmilewitz etc) On p 174 we have described such a case in which erythro leukæmia developed



FIG 49 Focal arrangement of paramyeloblasts (↗) in the spleen (analogous to metastasis of a neoplasm) in the early stage of a case of acute paramyeloblastic leucosis (section from embedded spleen puncture material)

In addition to haploid mitoses other abnormalities of division we have observed in the spleen marrow and not rarely in the peripheral blood (Andres and Shiwago) multipolar mitoses and clumping fragmentation or a splintering off or thickening of the chromosomes

Diagnostic Significance Spleen puncture plays no part in the diagnosis of paramyeloblastic leucoses Sternal puncture is far more useful and may indeed allow of a diagnosis being made before

myeloid cells are numerous with predominance of erythropoietic elements associated with high erythroblast values in the marrow *As the erythroblasts in spleen films from chronic myeloid leucoses may be as high as 33 per cent only cases in which there is also great proliferation of erythroblasts in the marrow should be classified as erythroleukæmias*

We have 3 cases in which spleen puncture was performed (see Table 22 Case 11–13)

Case 11 A F female aged 42 housewife

Increasing weakness for some years In April 1943 enlargement of the spleen and liver was noted hæmoglobin 52 per cent leucocytes 64 200 with many myelocytes and erythroblasts Admitted to hospital June 9th 1943

On examination Enlarged glands Spleen firm and palpable 17 cm liver somewhat enlarged 12 cm. in the parasternal line B S R 12 mm protein 6 0 prothrombin 60 per cent Takata negative Weltmann 0 2 serum iron 65 gamma

Blood Picture Red corpuscles 3 5 million hæmoglobin 70 per cent leucocytes 43 300 reticulocytes 2 3 per cent normoblasts 290 *myeloblasts 7½ per cent premyelocytes 2⅓ per cent myelocytes* 9 per cent metamyelocyte 7 per cent neutrophiles staffs 10⅓ per cent segmented 27⅓ per cent basophile 12⅓ per cent monocytes 43 per cent and lymphocytes 8 per cent platelets 220 000

Sternal Puncture (June 23rd 1943) Erythroblasts 4 2 per cent myeloblasts 3 per cent myelocytes 29 3 per cent neutrophiles 47 3 per cent

Arsenic was administered and the leucocytes decreased

November 1943 Gradual increase of erythroblasts in the blood (November 24th 1943) Red corpuscles 2 5 million hæmoglobin 65 per cent leucocytes 19 100 erythroblasts 3 250 myeloblasts 4½ per cent myelocytes 2½ per cent

Spleen Puncture (December 28th 1943) See Table 22 Great increase of erythroblasts (59 per cent) while there were 38 3 per cent in the sternal marrow with 16 9 immature granulocytes March 23rd 1944 second spleen puncture (see Table 22)

The leucocytes were kept between 10 000 and 13 000 by arsenic until death occurred on April 19th 1944

Autopsy confirmed the diagnosis of chronic myelosis with granulocytic and erythroblastic infiltrations of liver spleen glands and a distinct increase of the erythroblasts even in the bone marrow

This was thus a typical case of chronic erythroleukæmia in which there were 3 250 normoblasts in the blood and up to 59 per cent of erythroblasts in spleen puncture so that the 4 per cent of myeloblasts and 11 per cent of myelocytes were almost overshadowed (Fig 49)

Proliferation of erythroblasts was also well seen in the marrow where red cell formation is diminished in ordinary cases of leukæmia There was distinct increase of basophilic erythroblasts unlike the condition in myelocytic leucoses

In the second case (Number 12) we were dealing with a woman of

5 ERYTHROLEUCOSES AND ERYTHROBLASTOSES

We owe the concept of erythroleukæmia to di Guglielmo who was the first to publish reports of the disease Since then a number of cases of concomitant proliferation of the erythropoietic and leucopoietic tissues has been recorded Sometimes the former becomes more and more pronounced so that a case of chronic erythroleukæmia may end as one of acute erythroblastosis (*vide* Heilmeyer and Schoner)

Pure erythroblastoses (erythræmia acuta of di Guglielmo) are probably much rarer than are erythroleukæmias which form 2 per cent of the leucoses seen in our clinic In the literature up to 1939 (Moeschlin (1)) we found 5 proven cases, and further search has revealed records of 9 more (Israels 1939 Barcaglia, 1939, Quattrin 1939 Roth 1940 Nabholz 1942, Lanza 1940 Duvois 1941 Kienle 1943 Poli 1947, Denolin 1949) As far as we are aware there are no records of spleen puncture in pure erythroblastoses

The cases published by Weil (3) in 1938 under the name crypto erythroblastoses were certainly not examples of genuine erythroblastoses The great splenomegaly and the increase of megakaryocytes in spleen films make clear that some of Weil s cases were either aleukæmic or sub leukæmic examples of chronic myelocytic leukæmia In other cases he appears to have been dealing with a special form of reaction to infection especially tuberculosis of the spleen with many erythroblasts and myeloblasts in spleen films

In one of his female patients there had been great splenomegaly for 16 years with 6 5 million red corpuscles 35 000 leucocytes and occasional erythroblasts and myeloblasts while spleen films contained large numbers of immature granulocytes and erythroblasts Autopsy revealed typical tuberculosis of the spleen in which Koch s bacilli were demonstrated

It is not clear whether this was an example of primary tuberculosis of the spleen (with consequent formation of hæmopoietic foci) as in our own cases or whether tuberculosis was an intercurrent infection in the course of chronic myeloid leukæmia The latter is a fairly common terminal event which we have been able to observe three times

The fact that the leukæmic blood picture persisted after splenectomy in Weil s cases is strong evidence against their being of inflammatory nature We much prefer the term myeloid reaction associated with infective splenomegaly to crypto erythroblastosis which we consider totally misleading (see p 108) Nordenson (1946) has recorded 3 cases which he reckons as examples of the disease described by Weil In 2 cases spleen puncture films contained many erythroblasts and myelocytes but as neither splenograms nor myelograms are given exact classification is not possible

Spleen punctures in erythroleukæmia (excluding Weil s (3) dubious cases) have been recorded by Bianchi (1939) Forconi (1939) and Heilmeyer and Schoner (1941) They all agree that

ment had to be given because of the discomfort due to the enormous size of the spleen although the characters of the blood picture itself would not have necessitated irradiation This patient has been under our observation now for five years and if one were not acquainted with the earlier findings one would now regard the condition as being pure chronic erythroblastosis

This case is of particular interest inasmuch as it demonstrates the close relationship between polycythæmia chronic myelocytic leucoses and erythroleukæmia so that all these three diseases can be regarded as being variants of one and the same pathological process In our cases of erythroleukæmia we found many degenerative changes in the erythroblast mitoses *e g* complete pyknosis of the chromosomes and cells with detached fragments of chromosome material etc These changes are presumably due to the leukæmic nature of the affected cells but it has to be remembered that similar alterations have been described by Fieschi and also Kienle in cases of toxic damage to the marrow

In every case there was a distinct excess of mitoses in the polychromatic erythroblasts while the mitotic curve showed an excess of monaster types This does not differ appreciably from the proportion of erythroblast mitoses of the mitotic curve in normal marrow (Fieschi Kienle)

It may be said therefore that spleen puncture in our 3 cases of erythroleukæmia showed well marked proliferation of erythroblasts particularly of the immature forms the numbers of which exceeded those of the white cells although these also showed overgrowth

Spleen puncture in these cases is likely to be of diagnostic value only in those cases in which no marrow can be obtained by sternal puncture and in which the presence of osteosclerosis might arouse a suspicion of a myeloid reaction due to the formation of extra medullary myeloid foci The number of erythroblasts in spleen puncture in cases of non leukæmic origin never exceeded 20 per cent while the number of myelocytes in inflammatory conditions was never higher than 3 per cent In osteosclerotic anæmias (p 66) the myelocytes might be as high as 25 per cent but the lymphocytic structure of the organ (up to 60 per cent) was preserved as compared with the striking diminution of lymphocytes in erythroleucoses *It is probable therefore that purely reactive myeloid hyperplasias can be distinguished from leukæmic diseases by this quantitative relationship*

6 EOSINOPHILE BASOPHILE AND MEGAKARYOCYTE LEUCOSES

As in the case of erythroleucoses it is obvious that there are no pure leukæmic proliferations of a single cell type Thus there are innumerable variants between typical myelocytic leucoses and cases

61 who showed well marked enlargement of the liver and spleen In the blood the leucocytes reached 20,000 with 1,570 erythroblasts and 2 per cent of immature myelocytes In spleen puncture there were 41 *per cent of erythroblasts* but only 13 6 per cent of immature granulocytes Unfortunately we were never able to aspirate any bone marrow This failure was apparently due to patchy osteo sclerosis which was well shown by X rays At autopsy hyperplastic red marrow was found in the long bones and the histological appear ances were typical of erythroleuk emia *In a third case* (Number 13), a man of 54 presented an enormous swelling of the spleen (42 cm) which extended into the right iliac fossa The red corpuscles were 6 2 *million with 118 per cent hæmoglobin* the leucocytes at this time were 15 000 with 4 5 per cent myelocytes and 3 800 erythroblasts

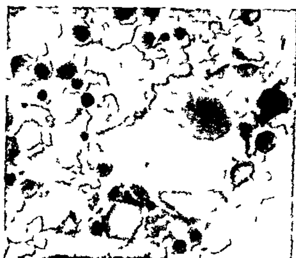


FIG 49 Erythroleukæmia with large numbers of erythroblasts and one megakaryocyte (spleen puncture)

Spleen puncture showed general infiltration of the spleen with erythroblasts of which there were 53 5 per cent and only 20 per cent of myeloid cells (Table 22) *The sternal marrow showed a mainly erythroblastic hyperplasia with 283 erythroblasts to every 100 white cells* (73 per cent erythroblasts) As the disease developed the erythroblasts became more numerous and less mature Treatment with arsenic appeared to inhibit the abnormal erythroblasts and in spleen puncture they fell from 54 per cent to 23 per cent in a month while the spleen diminished in size from 42 cm to 27 cm Within 5 months the spleen became enlarged again while the erythroblasts in the splenogram rose to 71 per cent and no myelocytes could be found Careful irradiation which had to be repeated every 6 or 8 months succeeded in reducing the size of the spleen and in diminish ing the percentage of erythroblasts in it to about half The treat

In some cases of reticulosis abnormal globulins similar to those found in association with myeloma are produced by the neoplastic cells (p 127) A hyperproteinæmia may result and the Takata cephalin and cadmium reactions can be positive while the B S R is greatly increased and in some cases there is a pathological electro phoretic pattern (Wuhrmann and Wunderly)

Waldenstrom (1948) has recently described a new disease



FIG 50 *a b c* *Reticulosis of spleen and gland* (Female aged 46)
(*a*) Rather large lobulated cells with small indistinct nucleoli a compact chromatin is characteristic of the cells of the reticuloses (Spleen puncture)
(*b*) Mitosis in such a cell rather short thick chromosomes
(*c*) *Phase contrast picture* The absence of distinct nucleoli is characteristic This should be compared with the cells of lymphosarcoma and other sarcomas (Zeiss phase contrast microscope 1 000)

having a distinct difference to myeloma associated with macro globulinæmia and infiltration of the marrow with small cells resembling lymphoid reticulum cells while a protein of high mole cular weight was present in the blood

These observations indicate how important is the part played by the reticulo endothelial system in the form of certain protein sub stances It seems to us probable that those reticuloses in which there is distinct formation of reticular fibrils are usually not asso ciated with changes in the serum proteins The power of synthesis

in which there is great predominance of one particular cell type

We have seen cases in which it has only been during the course of the disease that cells of eosinophile or basophile series have greatly overwhelmed those of other types

From the point of view of differential diagnosis the only cases that present difficulty are those of the syndroma of chronic eosinophilia with splenic enlargement Heilmeyer has shown that leukæmic eosinophilia can be distinguished from the non leukæmic type by the action of urethane in the former Also we have no experience of spleen puncture in eosinophile leukæmias and have been unable to find any in the literature We should assume that spleen puncture would be useful in diagnosis and we should expect to find many immature eosinophiles myelocytes and distinct reduction of lymphocytes (see also p 133) Perhaps in the non leukæmic type of splenomegalia with chronic eosinophilia the number of lymphocytes would still be elevated in comparison with the leukæmic form corresponding to our findings in osteosclerosis and myelofibrosis

We also have no personal knowledge of spleen puncture in true megakaryocyte leucoses and none appear to have been recorded We should however expect to find many of the more immature precursors of these cells The same is presumably true of the rare condition of thrombocytosis (thrombocythæmia of Mortensen and Hemmeler) which may also be associated with splenomegaly

7 PRIMARY MALIGNANT RETICULOSES AND RETICULO ENDOTHELIOSES

It is necessary to discuss the concept of primary malignant reticuloses because of the confusion of names used in the literature We include these conditions in our section on hæmoblastosis because by the term primary malignant reticuloses we mean a neoplastic condition in which there is a proliferation of undifferentiated cells of the reticulo endothelial system which does not remain localised and in which such cells may appear in the blood it is therefore possible to distinguish leukæmic and aleukæmic reticuloses (Uehlinger)

Most of the publications that have appeared on this subject have been on a pathological and anatomical basis without examination of the marrow glands or spleen by the method of puncture It is however impossible by the anatomical method to separate a para myeloblastic leucosis from a small celled sarcoma or a reticulosis except in those cases where there is distinct formation of reticular fibres Even so genuine reticuloses do occur in which the power of forming fibrils has been lost The presence or absence of such fibrils depends on the type of proliferating reticulo endothelial cell and its degree of differentiation

system can occur in infective conditions (Uehlinger Deelmann) It is therefore reasonable to speak of primary neoplastic malignant reticulosis and on the other hand, also of infective reactive secondary reticulosis (Uehlinger Glanzmann) In one case of Still s disease (p 98) we observed such a secondary reticulosis in which there was great increase of lymphoid reticulum cells in the sternal marrow (99 to every 100 granulocytes) while plasmacytoid reticulum cells were greatly increased (14 4 per 100 granulocytes) In the spleen only the plasmacytoid reticulum cells were increased the other reticulum cells showing no changes The problem of infective reactive reticulosis has been discussed in the section on infectious *mononucleosis* (p 87)

Deelmann would place many of the cases that we have called primary reticulosis into a separate group which he classes as being reactive in nature and places between the true inflammations and the true neoplasias It is of course always possible as in the case of leucoses that certain so called neoplastic forms are really due to infection by a virus

The numerous cases of so called monocytic leukæmia that have been published show how much care is needed in separating this condition from the primary reticuloses and also that it is not sufficient to depend upon the blood picture and the histological appearances Thus by sternal puncture in all our cases we were able to detect the origin of these cells from typical promyelocytoid para myeloblasts (Moeschlin and Rohr (3)) and not from reticular endothelial cells We do not however doubt the existence of a true monocytic reticulosis originating from the reticulo endothelial system We would however emphasise as we have done with Rohr that the diagnosis of a genuine reticulo endothelial monocytic leukæmia is never convincing in the absence of sternal puncture

It has already been mentioned in the discussion of lymphatic leukæmia that some of the cases are really reticuloses especially those with a remarkable cell polymorphism and atypical cell forms in those forms in which there is a close resemblance to the structure of lymphocytes when definite diagnosis is impossible These conclusions are not practically important because the general view is that these diseases are neoplastic in nature and dedifferentiation would then affect the reticulum cells and the elements transitional between them and the lymphatic myeloid and erythroblastic elements (Apitz Rohr Heilmeyer) But in all such cases an increased B S R and other changes in the blood proteins suggest a reticulosis

Storage diseases of the reticulo endothelium will be discussed in a separate section

We have records of 7 cases of primary reticulosis in which spleen puncture was performed In all of these it was possible to exclude lymphatic and myeloid leucoses because of the structure of the

ing proteins by such cells manifests itself in the formation of a more or less fixed protein product, whereas in the reticuloses in which fibrils are not formed changes are to be expected more frequently in the blood proteins because these cells may under certain circumstances, deliver their proteins into the blood stream

In our opinion a diagnosis of true reticulosis is most likely to be made by examination of cells in films of marrow glands spleen and perhaps liver, while in a few cases the characteristic cells may be found in the blood There is no doubt that this method is much less misleading than that of fixation with the consequent shrinkage of cells

Morphology The cells of primary reticulosis as seen in films have a fine granular but remarkably close and compact chromatin as compared to those of lymphosarcomas or paramyeloblastic leucoses (Figs 40 50 *a*, 51 *a c*, 52 *e f* and 53) The nuclear structure bears some resemblance to that of myeloma cells and also that of glandular fever cells The nucleus is more or less round and often shows a tendency to indentation or even lobulation As compared with paramyeloblasts and lymphosarcoma cells the nucleoli are smaller and scantier (usually 2–4) and may even be absent The cytoplasm is often very narrow (Figs 40 and 51) but occasionally is relatively bulky (Fig 53) and according to the stage of maturation shifting from intense basophilia to a greyish colour and as in the case of other neoplastic cells there may be numerous vacuoles In Case 5 the cells had relatively dark blue cytoplasm and closely resembled myeloma cells whereas in Case 3 (Fig 50) their appearances were those of genuine large atypical reticular monocytes In Case 6 there was some resemblance to atypical lymphocytes

Mitoses are usually relatively numerous The chromosomes being rather thick and nearly touching one another at an acute angle (Fig 51 *e*)

In some cases even examination of puncture fluid does not permit of a definite classification of the cells and these cases have to be recorded simply as hæmoblastoses or leukoblastoses Sometimes the striking size and density of the nuclei or the extreme basophilia of the cytoplasm enables a diagnosis of reticulosis to be made especially when the sternal marrow shows no proliferation of these cells If however there are no distinctive structural changes and if all the blood forming tissue is affected it may be impossible to distinguish the condition from an ordinary paramyeloblastic leucosis or sarcomatosis Changes in the blood proteins then may point to the diagnosis of reticulosis

The finding of reticulo endothelial cells in the blood even if accompanied by proliferation of such elements in the tissues is not sufficient grounds on which to base a diagnosis of primary reticulosis because reactive proliferations of the reticulo endothelial

of firm consistence The tonsils were not enlarged the spleen was enlarged to percussion but was not definitely palpable The liver was not enlarged W R negative B S R 41 mm serum protein 5 4 Takata reaction negative Weltmann reaction normal serum iron 80 bilirubin 1 9 X rays of the pelvis and stomach showed no abnormality but there was a mass in the region of the hilum of the right lung Fractional test meal showed histamine refractory achylia

Blood Picture Hæmoglobin 51 per cent red corpuscles 2 4 million reticulocytes 2 2 per cent leucocytes 4 300 myelocytes ½ per cent staffs 17 5 per cent polymorphs 33 per cent eosinophiles 0 basophiles 1 5 per cent monocytes 21 per cent lymphocytes 12 5 per cent *tumour cells 15 per cent* ! platelets 32 500 Red corpuscles showed anisocytosis and polychromasia

Structure of the Tumour Cells These showed considerable variation in size some being as small as old lymphocytes while others were as large as neutrophiles The cytoplasm was quite narrow and was pale blue and many cells appeared to consist of naked nuclei *The chromatin was strikingly close and homogeneous quite unlike that of myeloblasts or young lymphocytes* Some of the nuclei showed distinct indentations and lobulations the latter always lying close together and at first glance appearing as if they were splits in the nucleus (Fig 40) Unlike the cells of lymphosarcoma or paramyeloblasts very few of these elements possessed one or two indistinct nucleoli The smallest of these cells could not always be distinguished from old lymphocytes but as a rule the striking density of their nuclei enabled the distinction to be made Even in the peripheral blood occasional mitoses were seen (Fig 51 *e*)

TABLE 30

Hæmogram					Splenogram														
Leucocytes (in 1 000)	Neutrophiles staff	Neutrophiles segmented	Lymphocytes	Tumour cells	Macrophages	Plasma et retic cells	Pulp cells	Erythroblasts	Myelocytes	Neutrophiles staff	Neutrophiles segmented	Basophiles	Monocytes	Lymphocytes small	Lymphocytes large	Lymphocytes total	Plasma cells	Tumour cells
3 6	25	39	12½	1½	0 3	0 1	4 5	0 2	0 7	7 1	70	0 7	7 5	56 4	5 8	62	0 6	9 1

Spleen Puncture (Table 30) In addition to the cells of normal spleen puncture there were a few erythroblasts (0 2 per cent) and myelocyte (0 4 per cent) together with 9 1 per cent of the abnormal cells already described On the whole these cells were rather larger than those in the blood and their lobulation was more striking In sections from spleen puncture material it is possible to recognise the presence of large numbers of cells with indented and lobulated nuclei composed of rather dark chromatin These almost fill the pulp and only a few lymphocytes myelocytes and erythroblasts could be seen lying between them (Fig 51 *b*) The reticular fibrils are greatly increased

Sternal Puncture Macrophages 0 2 per cent lymphoid reticulum

characteristic cells and sometimes by the distribution of reticulum fibrils or by changes in the serum proteins A typical case is recorded in detail



FIG 51 Primary acute reticulosis (reticulo-endothelioses)

(a) Typical pathological reticulum cells in spleen films (see also coloured Plate II Fig 40)

(b) Section from spleen puncture material showing increased cellularity and increase of reticulum fibrils

(c) Pathological reticulum cells (Gland puncture films from a case of primary acute reticulosis)

(d) Section from an excised lymph gland The normal structure has completely disappeared tumour cells infiltrated the capsule and there is considerable increase of reticulum fibrils

(e) Blood film Mitosis of a typical pathological cell of the same type as in the spleen and glands

Case 1 B K Housewife aged 74 admitted to hospital August 25th 1943 with a history of rheumatism at the age of 43 and of recently increasing fatigue and occasional fainting attacks

On examination there was distinct pallor and aortic incompetence practically all the lymphatic glands were about the size of hazel nuts and

system including the spleen was mainly affected while the liver was only slightly involved and the marrow remained normal (Fig 52 *a b*) The proliferating abnormal cells were found in considerable numbers in the blood (650 cells per c mm *i e* 15 per cent of the total nucleated cells) The condition has however to be regarded as a subleukæmic form of reticulosis in which there was histological formation of reticular fibrils in the spleen and glands (Fig 51 *b* and *d*)

Clinically the case was characterised by gradually increasing enlargement of the spleen and particularly of the lymphatic glands accompanied by anæmia and reduction of serum protein The B S R was distinctly increased and X rays did not reveal changes in the bones

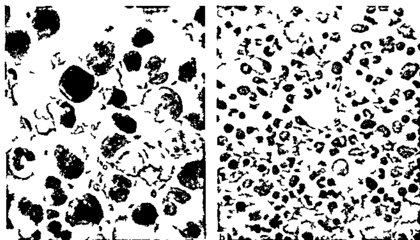


FIG 52 *a b* *Sternal marrow of primary acute reticulosis*
Note the normal marrow in contrast to paramyeloblastic leukæmia
(*a*) smear (*b*) section no increase of the reticulum

The condition of the marrow was such as to exclude a paramyeloblastic leucosis with certainty while lymphatic leukæmia could also be excluded because of the structure of the cells and the formation of reticular fibrils Furthermore the histological appearances in the spleen and liver were not those of lymphatic leukæmia Even the young cells had a strikingly close and homogeneous chromatin with distinct indentations and lobulations of the nucleus unlike those found in any case of lymphatic leukæmia

The fact that the pathological anatomist at the autopsy recorded the condition as being paramyeloblastic leucosis is explained by the fact that the details of the nuclear structure could not be recognised in post mortal and fixed tissues Especially the post mortal autolytic changes in the bone marrow made a correct examination difficult

cells 0 8 per cent tumour cells 4 per 100 white cells Erythro blasts basophile 6 4 per cent polychromatic 35 8 per cent myelo cytes 0 4 per cent myelocytes immature 8 2 per cent half mature 18 per cent mature 2 2 per cent metamyelocytes 6 per cent neutro philes staff 43 per cent segmented 7 4 per cent eosinophiles 2 per cent basophiles 0 6 per cent monocytes 3 per cent lymphocytes 9 2 per cent (Fig 52 *a*) This shows slight shift to the left with a few pathological cells of the same type as those found in the blood glands and spleen

In sections from the marrow puncture material it was found that the structure was practically normal and that there were no focal infiltrations (Fig 52 *b*) or increase of reticulum cells

Puncture of a cervical gland was difficult because of the dense con sistence In films no lymphocytes were found practically all the cells being of the pathological type already described but with even more striking variations in size and lobulation

Sections of an excised cervical gland showed that the cells formed a rather reticular but closely woven tissue The fibrils stained faintly red with Van Gieson and were well stained with silver In the peripheral parts the protoplasm of the cells were rather scanty while the nuclei were here rather larger than those of the cells in the centre There were many mitoses and occasional giant cells The tumour had broken through the fibrous capsule in several places

A histological *diagnosis* (Professor von Meyenburg) *of reticulum sarcoma was made* (Fig 51 *d*) Treatment consisted of repeated blood transfusions which produced slight improvement The leucocytes varied between 2 500 and 4 800 with 8–15 per cent of pathological cells The patient was discharged on October 23rd 1943 with the hæmoglobin up to 63 per cent This had fallen to 51 per cent by the middle of December when the glands had also increased in size She was re admitted on January 16th 1944 Her general condition was bad hæmoglobin 33 per cent red corpuscles 1 2 million leucocytes 7 400 1 normo blast per 200 white cells myelocytes 2 per cent staffs 15 5 per cent polymorphs 20 5 per cent eosinophiles 0 basophiles 0 5 per cent monocytes 12 per cent lymphocyte 18 per cent tumour cells 32 per cent The spleen was palpable and firm and all the superficial lymphatic glands were about the size of hazel nuts but in the right axilla there was a large hard swelling Transfusion did not lead to improvement and death occurred 2 days later

On autopsy (Professor von Meyenburg Pathological Institute University of Zurich) the majority of the lymphatic glands were enlarged but especially those of the upper part of the body and showed the same histological picture as described before Liver of 2 000 gm Infiltrations in Glisson s capsule around the central veins and also in the parenchyma The great majority of the cells were more or less round with relatively large nuclei and narrow cytoplasm The fibrils between these cells were very scanty and delicate A few of the large cells contained 2 nuclei and it was possible to observe transitions from indented to lobulated types

Spleen 1 080 gm the trabeculæ were delicate and the follicles were numerous Pale round cells with lobulated and sometimes double nuclei were seen in the follicles and in the congested pulp with the formation of many reticular fibrils A histological diagnosis of paramyeloblastic leukæmia was made

This was a typical case of primary reticulosis in which the lymphatic

was poor the liver and glands were not enlarged Blood red corpuscles 2 9 million hæmoglobin 49 per cent leucocytes 720 with 32 per cent neutrophiles (of which 22 per cent were staff forms) lymphocytes 56 per cent monocytes 12 per cent platelets 3 000 At this time no abnormal cells were found in the blood Sternal puncture showed distinct immaturity and signs of inhibition (hypersplenism) but no abnormal cells Serum protein 5 9 mg per cent The B S R was 7 mm which was surprising as there was high intermittent fever Blood culture was negative and neither penicillin nor sulphone had any effect

Spleen Puncture Many scanty normal lymphocytes and occasional myelocytes and erythroblasts there were 25 per cent of large reticular tumour cells showing great variation in shape and size As Fig 52 *c* shows these had a relatively large nucleus which was rounded also often showing indentation or lobulation The chromatin was delicate but very densely packed especially in the older forms The nucleoli were mainly small (3–4 in number) and were not as distinct and properly demarcated as in lymphosarcoma cells The cytoplasm was narrow and deeply basophil sometimes containing many small vacuoles The mitoses showed thick short chromosomes A few tripolar mitoses (Fig 52 *d*) and occasional multi nucleated cells were seen A diagnosis of retothel sarcoma or of reticulosis was made (In this case spleen puncture was performed in spite of the thrombocytopenia because the bleeding time was not prolonged)

Course After diagnosis had been made irradiation of the spleen was tried and after 4 treatments the temperature fell and in spite of the repeated irradiation the leucocytes increased somewhat and the general condition improved Then the pyrexia recurred and the inguinal and axillary lymphatic glands increased to the size of walnuts

Gland Puncture showed the presence of tumour cells of the same type as those in the spleen associated with almost complete absence of normal lymphocytes

Histological examination (Professor von Albertini Pathological Institute *University of Zurich) showed the structure of a round celled* sarcoma

The glandular enlargement nearly disappeared without further treatment and the general improvement was so great that the patient was discharged in February 1948 when the hæmoglobin was 88 per cent and the leucocytes 3 800 The spleen was now scarcely palpable (14 cm) and there were no enlarged lymphatic glands

He remained in good health until May 1948 when there was a relapse with fever leucopenia and swelling of the spleen and various lymphatic glands then a second spontaneous remission occurred and the patient was able to return to light work In July 1948 there was a relapse and the patient was readmitted to hospital The liver and spleen were distinctly enlarged but no lymphatic glands could be felt The leucocytes were 400–900 but rose to 3 700 with 53 per cent of tumour cells shortly before death No definite tumour cells were ever found in the bone marrow death occurred on September 6th 1948

Autopsy (Professor von Meyenburg Pathological Institute University of Zurich) Splenomegaly was confirmed and many enlarged abdominal lymphatic glands were discovered Histologically these showed severe changes but the capsule was intact All the sinuses were narrowed and the lymphatic tissue was completely replaced by abnormal tissue in which a few scattered lymphocytes could be found The characteristic cells

The diagnosis of the pathologist based on the excised lymph gland had pointed before (when patient was alive) to a reticulo sarcoma The findings demonstrate the difficulties arising for the pathologist in *the diagnosis of such cases and that only the close co operation* between clinician and pathologist enables a distinct evaluation of such cases

There is no doubt that this case should be included among the neoplastic proliferations of the reticulo endothelium but whether it has to be regarded as a ' retothel ' sarcoma with a leukæmic blood picture, in the sense used by Apitz, is not really of much importance

A summary of another of our cases is of interest at this point, because it shows that temporary partial remissions can occur in this neoplastic disease just as they can in the leukæmias

Case 2 K E *aged 25 policeman (November 1947 to September* 1948 †)

The disease started with weakness and fever in November 1947 when the spleen was found to be distinctly enlarged the general condition



FIG 52 *c d Reticuloses* (Spleen puncture)

(*c*) *Case* 2 male aged 25 The cells have intensely basophilic cytoplasm with compact chromatin and small indistinct nucleoli

(*d*) Abnormal tri polar mitoses from the same case

FIG 52 *e f g Case* 6 female aged 72 Large lobulated cells with strikingly dense chromatin (*e*) Spleen puncture (*f*) Blood film (*g*) Histo logical section of the spleen

was poor the liver and glands were not enlarged Blood red corpuscles ?9 million hæmoglobin 49 per cent leucocytes 720 with 32 per cent neutrophiles (of which 22 per cent were staff forms) lymphocytes 56 per cent monocytes 12 per cent platelets 3 000 At this time no abnormal cells were found in the blood Sternal puncture showed distinct immaturity and signs of inhibition (hypersplenism) but no abnormal cells Serum protein 5 9 mg per cent The B S R was 7 mm which was surprising as there was high intermittent fever Blood culture was negative and neither penicillin nor sulphone had any effect

Spleen Puncture Many scanty normal lymphocytes and occasional myelocytes and erythroblasts there were 25 per cent of large reticular tumour cells showing great variation in shape and size As Fig 52 *c* shows these had a relatively large nucleus which was rounded also often showing indentation or lobulation The chromatin was delicate but very densely packed especially in the older forms The nucleoli were mainly small (3–4 in number) and were not as distinct and properly demarcated as in lymphosarcoma cells The cytoplasm was narrow and deeply basophil sometimes containing many small vacuoles The mitoses showed thick short chromosomes A few tripolar mitoses (Fig 52 *d*) and occasional multi nucleated cells were seen A diagnosis of retothel sarcoma or of reticulosis was made (In this case spleen puncture was performed in spite of the thrombocytopenia because the bleeding time was not prolonged)

Course After diagnosis had been made irradiation of the spleen was tried and after 4 treatments the temperature fell and in spite of the repeated irradiation the leucocytes increased somewhat and the general condition improved Then the pyrexia recurred and the inguinal and axillary lymphatic glands increased to the size of walnuts

Gland Puncture showed the presence of tumour cells of the same type as those in the spleen associated with almost complete absence of normal lymphocytes

Histological examination (Professor von Albertini Pathological Institute University of Zurich) showed the structure of a round celled sarcoma

The glandular enlargement nearly disappeared without further treatment and the general improvement was so great that the patient was discharged in February 1948 when the hæmoglobin was 88 per cent and the leucocytes 3 800 The spleen was now scarcely palpable (14 cm) and there were no enlarged lymphatic glands

He remained in good health until May 1948 when there was a relapse with fever leucopenia and swelling of the spleen and various lymphatic glands then a second spontaneous remission occurred and the patient was able to return to light work In July 1948 there was a relapse and the patient was readmitted to hospital The liver and spleen were distinctly enlarged but no lymphatic glands could be felt The leucocytes were 400–900 but rose to 3 700 with 53 per cent of tumour cells shortly before death No definite tumour cells were ever found in the bone marrow death occurred on September 6th 1948

Autopsy (Professor von Meyenburg Pathological Institute University of Zurich) Splenomegaly was confirmed and many enlarged abdominal lymphatic glands were discovered Histologically these showed severe changes but the capsule was intact All the sinuses were narrowed and the lymphatic tissue was completely replaced by abnormal tissue in which a few scattered lymphocytes could be found The characteristic cells

were rather large, round oval or elongated elements which in places formed a network The nuclei were round or slightly indented usually being large and varying in the amount of chromatin they contained Very few mitoses were seen but a few cells had 2 or 3 nuclei These elements lay in contact with a network of very delicate fibrils most of which could be stained with silver also in addition there was an increase of collagen The arrangement of these reticulum was so diffuse that the systematic arrangements of normal lymphatic glands could no longer be detected

Spleen The appearances were very similar to those in the lymphatic glands The spleen tissue is replaced by the described cells and a network of reticular fibrils

The Bone Marrow The greater part was more or less normal but contained hæmorrhages and focal infiltrations of the typical tumour cells associated with increase of reticulum fibres

The other organs appeared normal

Summary The febrile attacks which occur in many reticuloses were well marked while the spleen and glands were also involved The bone marrow in the early stages was unaffected There were two marked spontaneous remissions so that it must be assumed that in this disease as in the leukæmias there is some unknown defensive mechanism against the number of proliferating neoplastic cells although this cannot permanently inhibit their growth

A particularly striking feature of this case was the extreme inhibition of the marrow which developed during each relapse and which was doubtless due to hypersplenism

Histologically in the spleen glands and in small foci in the marrow there was infiltration with proliferating reticular cells together with obvious formation of reticular fibrils

That these were definitely neoplastic elements not simply due to a reaction is shown by the gross abnormalities of structure of the characteristic cells (Fig 52 c and d) and also by the occurrence of abnormal mitoses The case also confirms our previous statement that when there is well marked formation of reticular fibrils there are generally no striking changes in the serum proteins

Of course reticulo endothelial proliferations may originate in the marrow and then it is very rarely possible to distinguish the condition from paramyeloblastic leukæmia But this distinction can be made if the cells are excessively large or if the chromatin is very different from that of myeloblasts (e g Kienle s case) It is also obvious when the cells have bulky basophilic cytoplasm as in plasma cell myeloma Kienle has recorded and illustrated a case in which the sternal marrow contained some enormous basophilic cells although none were found in the blood At autopsy there were miliary aggregations of these elements in the spleen glands and liver In cases in which the changes are less striking exact classification is not possible if the marrow is extensively involved

Dr Jucker (Medical Clinic Basel under the Directorship of

Professor Staub) has kindly given me access to material from another case (Case 3) of leukæmic reticulosis in which only the liver and spleen were affected while the marrow was normal Spleen liver and sternal punctures were carried out during life and a complete autopsy was performed Fig 53 *a* is from spleen films kindly given to us by Dr Jucker it shows large (18–35 μ) round cells with deeply basophilic vacuolated cytoplasm There is a resemblance to myeloma cells but the nuclei are larger denser and sometimes lobulated

I owe Case 4 to Dr Ludin (Medical Clinic Basel Professor Staub) It was that of a woman of 67 with a considerable degree of



FIG 53 (*a*) *Acute primary reticulosis of l er and spleen* Large atypical reti ulum cells in spleen puncture (Dr Jucker s case from which Professor Staub (Basel) kindly supplied spleen films)

insidious splenomegaly greatly increased B S R (103/122) and slight inhibition (probably splenogenic) of the marrow (leucocytes 3 700) In spleen puncture there were 96 per cent of lymphoid cells varying greatly in size and having a rather closely woven nucleus There was a distinct resemblance to the large lymphatic reticulum cells that are found in normal gland punctures At first we made a tentative diagnosis of aleukæmic lymphadenosis but in view of the greatly increased B S R concluded that it was a reticulosis The autopsy (Professor Werthemann University of Basel) revealed the typical features of a reticulosis with great increase of fibrils and proliferation of reticulum cells in the spleen and glands In places the capsule was penetrated by these elements This case

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cytoplasm and *no blue nucleoli* together with many eosinophiles Autopsy (Professor Uehlinger) after 4 years illness led to a diagnosis of lymphogranulomatoid reticulosis

Summary We have discussed primary malignant reticulosis as a neoplastic disease and our opinion is founded on our hæmatological and morphological observations Of course this does not exclude the possibility of the disease s being due to a virus infection as may also be the case in the leukæmias This is not the place to enter into all the arguments for and against the inclusion of the primary reticuloses among the neoplasias but the cases already described certainly seem to be explained in this way and this is further stressed by the undoubted resemblances between the cells of reticuloses and of sarcomas

The repeated punctures of spleen glands and marrow in our cases have demonstrated that in the early stages of primary reticuloses the disease is not systematic (We think that this is also the case in the leucoses) The initial neoplastic dedifferentiation seems to be localised (perhaps unicentric) later giving rise to metastases especially in lymphatic tissue (also although less frequently in the marrow liver and other organs) It seems as if these cells find the most suitable living conditions in their parent tissue It is because the pathologist only sees these cases in their terminal stage that he usually (Apitz) considers them to be examples of a system disease Although we cannot accept this idea in most cases we would not deny the possibility of an occasional pluricentric or systematised malignant change such as Rohr has suggested in a few cases (perhaps due to a virus infection) Even so not all reticuloses are genuine system-diseases We agree with Van der Meer and Zeldendrust (1948) that there are transitions between primary reticuloses reticulo sarcoma with localised growth the leucoses and Hodgkin s disease

We have devoted so much space to the reticuloses because they seem to be a group in which spleen and marrow puncture sometimes amplified by gland puncture are particularly useful for the gaining of an insight into the pathological process

XII SARCOMAS OF THE SPLEEN

1 LYMPHOSARCOMA

This quite commonly originates in the spleen (in 21 per cent of cases according to Sugerbaker) and then rapidly metastasises in other lymphatic tissues Sometimes malignant change starts in a lymphatic gland while the spleen is secondarily involved In the later stages the tumour cells may overflow into the blood giving rise to a leukæmic picture with 50 000 or more white cells We

was the only exception of marked serum protein changes and formation of reticular fibrils at the same time

This case clearly illustrates our earlier contention that there is so close a relation between the lymphadenoses and the reticuloses that a distinction may be rather difficult

There is also a close relationship between some atypical forms of Hodgkin s disease and the reticuloses this applies particularly to the so called Hodgkin sarcoma

Dr Buchler (Tiefenauspital Bern) has kindly given me spleen films from an important case (Case 5) A man of 21 showed areas of rarefaction and others of sclerosis in the skeleton There was only slight enlargement of the lymphatic glands which histologically showed only the picture of a chronic inflammation with plasma cells and many eosinophiles but no Dorothy Reed cells In the marrow there was an excess of eosinophiles together with small groups of lipoid cells Spleen puncture revealed the presence of up to 60 per cent of reticular tumour cells with deeply basophilic cytoplasm resembling glandular fever cells of varying stages of maturity Their great variation in size and the numerous mitoses clearly distinguished them from these elements An occasional cell of this type was found in the blood There was no excess of eosinophiles in the spleen

Although no autopsy has yet been performed it seems probable that this is a case of *primary reticulosis* in which there are resemblances to Hodgkin s disease It seems possible that the eosinophilia in the glands and marrow as well as the pyrexia are due to a sensitisation to some protein substance set free by the proliferating tumour cells We suggest a similar explanation for the eosinophilia and fever of Hodgkin s disease which we also regard as neoplastic (see Moeschlin and Schwarz)

A striking difference between the cells of reticulosis and those of Hodgkin s disease is the absence of the bluish violet nucleoli so characteristic of Sternberg cells For instance in Case 6 (a woman of 72) there was persistently high fever associated with distinct splenomegaly but no clinical diagnosis could be made The pathologist was able to demonstrate the presence of actively proliferating large cells with distinct lobulation in the spleen liver and lungs His diagnosis was atypical lymphogranuloma

A glance at Fig 52 *e f g* shows that these elements are in fact large lobulated reticular cells similar to those found in spleen puncture and that they are very different from those of Hodgkin s disease Even in the less mature forms no bluish violet nucleoli could be found Our view is therefore that this also is an example of genuine reticulosis

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have already discussed such a case in the section on acute lymphatic leukæmia (p 128) On the whole, lymphosarcoma differs from the relatively benign chronic lymphadenoses in its rapid and infiltrative growth

Clinically lymphosarcoma is characterised by enlarged, relatively soft glands sometimes by splenomegaly and usually by pyrexial



Fig 53 b c Lymphosarcoma of the spleen

(b) Spleen puncture Intensely basophilic cells Arrangement of the chromatin in masses is characteristic of the more mature types The large distinct nucleus is unlike that seen in the cells of chronic lymphatic leukæmia

(c) Phase contrast picture The large dark permanent nucleoli are very characteristic and are very different from the small numerous nucleoli found in lymphoblastoma and lymphatic leukæmia (Zeiss phase contrast microscope 1 000)

episodes In 2 cases there was extreme leucopenia probably due to splenopathic inhibition of the marrow The pyrexia is probably due to sensitisation to protein substances liberated by degenerating tumour cells this is similar to the state of affairs in association with other tumours and with Hodgkin s disease

Structure of Lymphosarcoma Cells There is a general resemblance to the younger forms of lymphatic cells but the lymphosarcoma cells differ from them by their great variation in shape and

size and especially by the presence of large vesicular nucleoli (Fig 53 *b c*)

The nucleus is roundish or oval often slightly indented and of very variable size but usually larger than that of a normal lymphocyte The finely granular chromatin is reminiscent of that of the lymphoblasts but is usually arranged in rather coarser masses while in the older forms it is denser and arranged in more sharply demarcated masses Some cases show rosette forms of the nucleus (Fig 53 *c*) The cytoplasm is usually narrow but varies from cell to cell it may be fairly bulky and is always intensely basophilic *The most characteristic features are the nucleoli* As is shown in Fig 53 *b* there is usually a very large rather pale nucleolus which is separated from the rest of the chromatin by a distinct membrane Occasionally there are two or three large nucleoli but more often there is one large one and several smaller ones Mitoses are numerous and the chromosomes are narrow lying at an acute angle

Phase Contrast Microscopy As we were the first to point out (Moeschlin (23 24) the large nucleoli are particularly well seen being strikingly dark This is a useful point of distinction from the smaller and paler nucleoli of young lymphocytes and from the cells of lymphoblastoma (Fig 53 *e*) In doubtful cases fresh preparations should always be examined with the phase contrast microscope

The typical characters of lymphosarcoma cells (variation in size narrow basophilic cytoplasm large nucleoli and numerous mitoses) can more readily be appreciated in gland puncture (Tischendorf 1 3 Stahel 1) and spleen puncture than in sections where it may be impossible to distinguish these cells from other somewhat similar ones

X ray Strunge has shown that structural changes can be found in lymphosarcoma cells as early as 5 hours after *deep X ray therapy* but that these are no longer detectable after 48 hours when active cell division again starts *This observation emphasises the need for daily treatment for long periods without any prolonged intermissions* a fact often mentioned by the radio therapists

Nitrogen Mustard This treatment is followed by rapid decrease in size of the spleen and glands Puncture 24 hours after intra venous injection of 2 mg showed evidence of an intense cytotoxic action viz rapid disintegration of pyknotic cells the presence of many macrophages filled with cell debris and virtual cessation of mitosis (Signs of damage to the process of cell division could often be seen *e g* clumping of the chromosomes etc)

2 FOLLICULAR LYMPHOBLASTOMA

(Brill Symmers Disease)

This disease is probably a neoplastic proliferation of the cells of the germ centres *i e* of those elements we have called large lymphatic reticulum cells

There is gradual enlargement of all the lymphatic glands the

masses being strikingly soft Histologically the appearances are those of enormous enlargement of the germ centres The course is long extending over several years while in the early stages, the general condition of the patient remains unimpaired (Salm)

That these tumours are more related to the reticular cells than to the lymphocytes is made evident by the existence of rare cases



FIG 53 *d e Lymphoblastoma folliculare (Brill Symmers disease)*
(*d*) Spleen puncture Numerous large polymorphic cells with indistinct but rather delicate chromatin somewhat resembling large lymphatic reticulum cells together with delicate epithelioid cells which mainly lie in groups of two or three
(*e*) *Phase contrast picture* The variations between these cells and those of lymphosarcoma are more striking by this method The numerous indentations of the nuclei sometimes in a rosette form and the numerous small nucleoli are clearly seen On the left there are two epithelioid cells

with a dysproteinemia (Rossier Spuhler) showing the same changes as seen in myeloma and primary malignant reticulosis

The spleen is almost invariably involved (Mayer 1939) Thus Baggenstoss (1940) found splenomegaly in 61 per cent of cases Stahel (1948) was the first to report on the appearances found by gland puncture in which he saw not only increase of germ centre cells but also occasional giant cells with 2 or 3 nuclei

There are no records of spleen puncture but owing to the

kindness of Professor Schinz (Rontgen Institute University of Zurich) we have been able to investigate a man of 70 in whom the diagnosis of this disease had been made by excision of a gland The spleen was enlarged (25 cm) and we were able to follow the effects of treatment with nitrogen mustard

Spleen Puncture A few normal lymphocytes granulocytes and plasma cells were seen but up to 84 per cent of the cells resembled those of the germ centres (our large lymphatic reticulum cells)

The nuclei are rather indistinct and consist of fairly dense chromatin composed of delicate connections There are 2–4 pale but small nucleoli (Fig 53 *d*) Many of the nuclei show delicate indentations and lobulation while a few present a rosette arrangement The cytoplasm is narrow and intensely basophilic the edge being irregular Mitoses are fairly numerous the chromosomes being delicate and at an acute angle

In addition to the more obvious tumour cells there are smaller types and occasional giant-cells with 2–3 nuclei and wide cytoplasm

The nuclei have a rather coarse meshwork and their structure is somewhat reminiscent of the epithelioid cells of tuberculosis and of the Dorothy Reed cells of Hodgkin s disease But unlike the latter they rarely contain bluish nucleoli (Fig 53 *d*)

The cells exactly resemble the giant-cells described in gland puncture by Stahel and are the same as those described by Kellert and Terplan in sections Presumably these malformations are due to failure of the cytoplasm to divide when the nucleus which is becoming pyknotic has done so

Gland Puncture The appearances were very like those in the spleen except that there were practically no normal lymphocytes plasma cells or granulocytes

Phase Contrast Microscopy Elsewhere (Moeschlin (24)) we have pointed out that this method of examination permits one to distinguish between these cells and normal ones as well as the abnormal elements of reticulosis lymphosarcoma and lymphadenosis In stained films confusion between these last two is almost inevitable *Phase microscopy reveals the cells of lymphoblastoma as having small dark nucleoli unlike the larger but equally dark ones of lymphosarcoma cells* They are distinguished from the lymphocytes of lymphadenosis by their great tendency to indentation and narrowings of the nuclei which may lead to the formation of rosettes (Fig 53 *e*) which are rare in other diseases of glands except in lymphosarcoma

Treatment with nitrogen mustard is followed by rapid decrease in size of glands and spleen together with gradual diminution of abnormal cells and reappearance of normal lymphocytes Many necrotic cells are present but one of the most striking features is reduction in the number of mitoses as well as the occurrence of atypical ones e g with condensed or swollen chromosomes

3 MELANOSARCOMA

The presence of metastases in other organs (skin liver etc) and perhaps the black colour of the tumours usually makes the diagnosis obvious The appearance of the typical pigmented cells

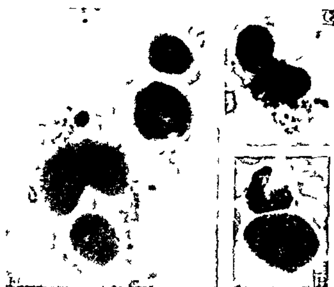


FIG 53 *f g* *Melano sarcoma*

(*f*) A group of epithelioid cells some containing melanin others not pigmented

(*g*) Cells containing much melanin

FIG 53 *h* *Retothel sarcoma* (Spleen puncture)

These cells show distinct blue or bluish violet nucleoli and can be confused with Sternberg cells The splenogram does not present the chronic inflammatory character that is found in Hodgkin s disease

is seen in Fig 53 *f g* Difficulty can arise if as is not uncommon the malignant cells contain no melanin

4 OTHER FORMS OF SARCOMAS

Unlike lymphosarcoma and lymphoblastoma in which the characteristic cells show some resemblance to lymphatic elements the cells of the undifferentiated sarcomas have a very different nuclear structure We prefer the term undifferentiated sarcoma to those commonly used by pathologists viz mixed celled sarcoma and giant celled sarcoma

We have records of 4 cases in which the growth probably did not arise primarily in the spleen but grew into it from the retroperitoneal tissues In one case spleen puncture revealed an ordinary chronic inflammatory picture At autopsy focal deposits of tumour were found but these had obviously been missed by the needle In the other 3 cases tumour cells were found

Retothel Sarcoma A man of 22 with a large firm spleen which moved with respiration Leucocytoses of 12 000 with some leftward shift but no abnormal cells in blood films Hæmoglobin 84 per cent B S R 30 mm

In spleen puncture scattered nuclei were found These were large and were composed of delicate but closely arranged chromatin with one or two distinct bluish nucleoli The cytoplasm was narrow and stained slightly blue (Fig 53 *h*)

The presence of bluish nucleoli suggested a diagnosis of retothel sarcoma or of Hodgkin s disease but the absence of plasmacytoid reticulum cells and eosinophiles was against the latter possibility Operation revealed a large retroperitoneal retothel sarcoma which had pushed the enlarged spleen forward

The presence of bluish nucleoli which has also been reported by Ludin seems to be a valuable feature in distinguishing the retothel sarcomas from the true reticuloses in which the puncture may in other respects be very similar

Undifferentiated Sarcoma In this very undifferentiated type (mixed celled and giant celled) of growth spleen puncture shows large groups of cells the cytoplasmic boundaries of which are not always recognisable Such a syncytial arrangement is shown in Fig 54 *a b* This is very different from the condition in reticuloses lymphosarcoma and lymphoblastoma in all of which only single cells or at the most small groups (in reticuloses) can be found

The chromatin is closer and smoother than in lymphosarcoma , and there is no tendency to an arrangement in fields such as is characteristic of lymphosarcoma Nucleoli are small and are sometimes difficult to detect Nuclear indentation may be seen but it never reaches the degree of lobulation found in some reticuloses

If these features of big syncytial arrangements with numerous mitosis can be found the diagnosis of sarcoma can be made but if only isolated cells are present it may be impossible

Fig 54 *a* is from spleen puncture on a woman of 62 who was admitted to the Clinic with high septic fever The B S R was 54 mm , hæmoglobin 60 per cent and a leucocytoses of 13 900 with distinct shift to the left There was an enormous hard tumour in the left upper abdomen

In spleen puncture there were a few blood cells and large syncytia in which the individual elements showed much variation in size The nuclei which were rounded were composed of closely woven but delicate chromatin The very narrow cytoplasm was *greyish violet in colour The nucleoli were small and could not be* recognised in all the cells

Autopsy revealed a large retroperitoneal sarcoma which had infiltrated the otherwise normal spleen

The third case was that of a woman of 21 in whom great spleno

3 MELANOSARCOMA

The presence of metastases in other organs (skin liver etc) and perhaps the black colour of the tumours usually makes the diagnosis obvious The appearance of the typical pigmented cells

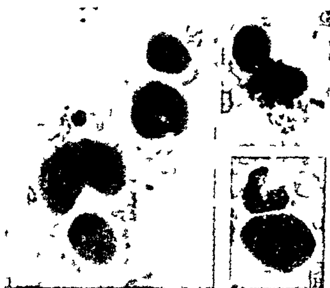


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(*f*) A group of epithelioid cells some containing melanin others not pigmented

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In spleen puncture scattered nuclei were found These were large and were composed of delicate but closely arranged chromatin with one or two distinct bluish nucleoli The cytoplasm was narrow and stained slightly blue (Fig 53 *h*)

The presence of bluish nucleoli suggested a diagnosis of retothel sarcoma or of Hodgkin s disease but the absence of plasma cytoid reticulum cells and eosinophiles was against the latter possibility Operation revealed a large retroperitoneal retothel sarcoma which had pushed the enlarged spleen forward

The presence of bluish nucleoli which has also been reported by Ludin seems to be a valuable feature in distinguishing the retothel sarcomas from the true reticuloses in which the puncture may in other respects be very similar

Undifferentiated Sarcoma In this very undifferentiated type (mixed celled and giant celled) of growth spleen puncture shows large groups of cells the cytoplasmic boundaries of which are not always recognisable Such a syncytial arrangement is shown in Fig 54 *a b* This is very different from the condition in reticuloses lymphosarcoma and lymphoblastoma in all of which only single cells or at the most small groups (in reticuloses) can be found

The chromatin is closer and smoother than in lymphosarcoma and there is no tendency to an arrangement in fields such as is characteristic of lymphosarcoma Nucleoli are small and are sometimes difficult to detect Nuclear indentation may be seen but it never reaches the degree of lobulation found in some reticuloses

If these features of big syncytial arrangements with numerous mitosis can be found the diagnosis of sarcoma can be made but if only isolated cells are present it may be impossible

Fig 54 *a* is from spleen puncture on a woman of 62 who was admitted to the Clinic with high septic fever The B S R was 54 mm hæmoglobin 60 per cent and a leucocytoses of 13 900 with distinct shift to the left There was an enormous hard tumour in the left upper abdomen

In spleen puncture there were a few blood cells and large syncytia in which the individual elements showed much variation in size The nuclei which were rounded were composed of closely woven but delicate chromatin The very narrow cytoplasm was greyish violet in colour The nucleoli were small and could not be recognised in all the cells

Autopsy revealed a large retroperitoneal sarcoma which had infiltrated the otherwise normal spleen

The third case was that of a woman of 21 in whom great spleno

megaly had led to a suspicion of Hodgkin s disease Spleen puncture showed the typical syncytia, no normal spleen cells being found Here again the undifferentiated structure of the nuclei was a striking feature (Fig 54 *b*)

It must be mentioned that the discovery of such groups of cells



FIG 54 *a b* *Polymorphic cell and giant celled sarcomas of the spleen* The large groups of cells (syncytia) in which no distinct cytoplasmic outlines can be detected is characteristic and is strikingly different from the condition in reticulosis lymphosarcoma and lymphoblastoma (*a*) Aged 62 (*b*) Aged 21

should also lead one to consider the possibility of hypernephroma and even of a pancreatic tumour

Other malignant tumours of the spleen are extremely uncommon (Lubarsch) Walther had shown that the rarity of metastases in the spleen is not due to a special defensive power of the reticulo endothelium but only to the peculiar arrangement of the circulation which tends to prevent entry of neoplastic cells

XIII CARCINOMAS AND CYSTS

In spite of the most careful clinical examination it is possible to mistake a mass in the left hypochondrium for an enlarged spleen when it is in fact a tumour (see Naegeli (2)) Of course spleen puncture will demonstrate this Thus in one of our cases puncture of what seemed to be an enlarged spleen showed typical tumour



FIG 54 *c d* *Hypernephroma*

(*c*) Film from puncture (A tumour was mistaken for an enlarged spleen) The typical appearance is that of groups of cells with rather bulky foamy and round dense nuclei which show considerable variations in size.
(*d*) Histological section of the same puncture material

cells in films (Fig 54 *c d*) This was later shown to be a hypernephroma *Weil has reported a similar case I am grateful to Dr* Vegh (of Szeged) for a further example

Structure of Hypernephroma Cells These are strikingly large but variable (15–80 μ) The round nucleus is relatively small (10–24 μ) and is composed of dense chromatin in which a small nucleolus can sometimes be seen The cytoplasm sometimes resembles a honeycomb but is usually a homogeneous pale violet or reddish violet colour Rarely some blackish pigment is present in the cells Cells with 2 or 3 nuclei may also occur The typical features are the occurrence of the cells in fairly large aggregations and the great variation in size of their nuclei and cytoplasm (Fig 54 *c d*) The latter feature also distinguishes them

from smaller cells of the pulmonary alveoli to which they have a distinct resemblance (Fig. 22 *d*)

In one case we found undifferentiated carcinoma cells and autopsy showed that the cells were divided from a carcinoma of the pancreas

Cysts *A woman of 35 had a large firm tumour in the left upper* abdomen which radiographically, appeared to be an enlarged spleen Radiotherapy produced no improvement indeed there was slow *but progressive increase in size Puncture was therefore performed* and sero sanguineous fluid, under pressure was obtained No microscopic signs of hydatid were found and operation (Professor Clairmont) revealed an enormous pancreatic cyst If therefore, spleen puncture results in such a fluid, it is necessary to investi gate its diastase content which we unfortunately did not do

Weil (1) recorded 2 cases in which a sero sanguineous fluid was obtained One proved to be a dermoid cyst of the spleen while the other *was a cystic angioma of the left lobe of the liver Other causes of enlarge* ment of this lobe must be borne in mind puncture will show the presence of liver cells with bulky finely granular cytoplasm and a small round nucleus (Stahel (3))

E DEGENERATIVE CONDITIONS OF THE SPLEEN

XIV AMYLOIDOSIS

We have punctured one case of almost certain amyloidosis (congo red test positive) but the dense consistency of the organ *prevented us from obtaining any spleen substance In such cases* sections would probably establish the diagnosis but examination of films would not be likely to do so

F STORAGE DISEASES OF THE SPLEEN

XV GAUCHER S DISEASE

Spleen puncture has often been used for diagnosis *e g*, by Sokolowski (1932) Potter and McRae (1933) Pick (1933) Merklen (1933) Lowinger (1935) and Eppinger (1939) Since the discovery *of Gaucher cells by sternal puncture (Lowinger Klima Rohr etc)* spleen puncture has become of less importance If as is sometimes the case sternal puncture fails to reveal the characteristic cells *(Pittaluga and Rof Barchasch and Gurin Renard) spleen* puncture is indicated

We have records of 4 cases (including 2 brothers) in which these *cells were found (2 of the cases were under Professor Loffler)*

The cells are very large (30 or 40μ up to 60μ) round or oval

elements with bulky cytoplasm and one or more nuclei lying near the edge (Eppinger *et al*) (Fig 55 *a*)

The reddish or violet cytoplasm is not sharply demarcated from the background and stained with Giemsa has an appearance resembling crinkled tissue paper This results from the network that remains after the lipoid (kerasin) has been extracted by alcohol The older cells are almost colourless and are often seen as blank spaces between more obvious cellular elements while younger cells are distinctly basophilic The small round nucleus usually lies at the periphery of the cell the chromatin being arranged closely but in a coarse network up to four

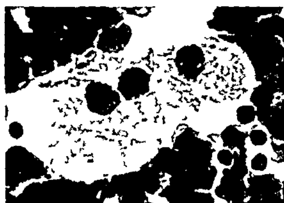


Fig 55 *a* Gaucher cells, some with 2 nuclei

nuclei may be present in one cell Only di Guglielmo (3) has recorded the presence of Gaucher cells in the peripheral blood

Unlike Niemann Pick cells (Fig 55 *b*) Gaucher cell do not contain distinct cytoplasmic vacuoles

TABLE 31

Gaucher s Disease

	1	?		1	?
Hæmogram			Plasmacyt retic cells	0 6	—
Hæmoglobin ( )	82	—	Pulp cells	0 2	—
Red corpuscles (in mill)	3 984	—	Erythroblasts	0 7	—
Thrombocytes	47 800	—	Myelocytes	1 6	1·0
Leucocytes	5 400	—	Neutrophiles staff	14 6	2 8
Myelocytes	1½	—	Neutrophiles segmented	26 8	4 6
Neutrophiles staff	8½	—	Eosinophiles	2 6	—
Neutrophiles segmented	69	—	Basophiles	0 5	—
Eosinophiles	1	—	Monocytes	5 8	—
Monocytes	3	—	Lymphocytes young	? 2	1 4
Lymphocytes	17	—	Lymphocytes old	41 8	81 0
Plasma cells	—	—	Lymphocytes total	44 0	82 4
			Plasma cells	0 2	—
			Gaucher cells, young	0 3	0 4
Sp enog am			Caucher cells old	2 1	8 8
Macrophages	—	—	Gaucher cells total	2 4	9 2

The presence of many such elements in the spleen puncture points distinctly to Gaucher s disease In Section I we have mentioned the occurrence of a few large lipophages in spleen puncture in inflammatory conditions (Fig 20) There is a special difference in size the lipophages of chronic inflammation were never more than 30 μ and we have never seen more than 8 of them on one slide In Gaucher s disease instead there may be 2 4-9 2 per cent of typical (larger) Gaucher cells (Table 31)

XVI NIEMANN PICK DISEASE

In children the rare Niemann Pick disease must be considered Here, phosphatides are laid down in the cells, and death occurs quite early Professor Willi (Sauglingsheim, Zurich) kindly allowed



FIG 55 *b* *Niemann Pick disease*

The typical appearances of these storage cells is that of a distinct fine network in the cytoplasm as compared with the less distinct structure of Gaucher cells (Male aged 1½ years the patient of Dr Willi of Zurich)

us to study a case in a child of 1½ years Spleen puncture showed normal cells together with 2 8 per cent of storage cells similar to those seen in sections by Pick and in sternal marrow smears by Esser Kienle and Bessis

Structure Although the cells are very like Gaucher cells they are less homogeneous in stained films The cytoplasm is completely filled with distinct small vacuoles (Fig 55 *b*) so that there is a close resemblance to fat macrophages (Fig 16 *a–d*) although of course the latter are always scanty while the former occur in groups and are more numerous

A peculiar form of xanthomatosis of the spleen occurring in a child aged 6 years with deposits of lecithin and cephalin has been described by Dreyfuss and Fishberg

In *the Schuller Christian disease* there is deposition of sterols

(and raised blood cholesterol) but the spleen is not usually enlarged (Eppinger (2)) Quattrin has described the typical cells in bone puncture and found that they differ from Gaucher cells by the reticular structure of their cytoplasm

Glycogen storage disease is rarely accompanied by splenomegaly *Hæmochromatosis* has been mentioned in the section on hepatic cirrhosis

G SPLENOMEGALY OF UNCERTAIN ÆTIOLOGY

Here we shall first discuss persistent eosinophilia with spleno megaly and then two personal observations on what may well be previously unknown maladies

Persistent Eosinophilia with Splenomegaly A number of authors have described cases with great eosinophilia accompanied by splenic enlargement (Cremer 1939 Tischendorf 1940 Meyer 1942) We have not seen a case but the literature leads us to assume that this is only a *syndrome* in which are included familial eosino philia (Stewart Atmar) so called eosinophil leukæmias (Thom sen and Plum) some unrecognised cases of Hodgkin s disease parasitic infestations and some chronic infective allergic condi tions such as Loffler s eosinophil mural endocarditis Doubtless combined spleen and marrow punctures will gradually clarify the position In one case Heilmeyer demonstrated the leukæmic nature of an eosinophilia by inducing a return to normal numbers after urethane It is possible that in cases of non leukæmic origin the *number of lymphocytes is not reduced as much as in spleens* of leukæmic origin which in the first case also contains probably many immature eosinophiles

Splenomegaly with Intense Lipæmia We have records of one such case in which even prolonged clinical observation did not *succeed in explaining the condition*

D A aged 28 complained of fatigue and lumbar pain in the autumn of 1941 He was admitted to hospital on February 15th 1942

General condition poor skin pale no enlargement of glands or liver Spleen distinctly palpable rather soft and slightly tender on pressure *Urine no red corpuscles or doubly refractile substances* a trace of albumin Esbach 0 25 / Blood pressure 130/100 Renal func tion tests normal B S R 6 mm W R and Brucella agglutination test negative serum protein normal 6 5 bilirubin 0 58 sodium chloride 602 calcium and serum phosphatase normal Takata negative Weltmann prolonged to 0 15 Persistent lipæmia (macroscopically visible) Fatty acids *greatly increased 1 600–3 600 mg per cent cholesterol high 324* esters 48 prothrombin reduced to 70 per cent but rose to 100 per cent after Synkavit B M R + 20 per cent No radiographic changes in bones

Blood Hæmoglobin 110 per cent red corpuscles 5 04 million

reticulocytes 1 6 per cent leucocytes 6 900 metamyelocytes ½ per cent staffs 13½ per cent segmented 59½ per cent eosinophiles 1½ per cent, basophiles ½ per cent monocytes 2½ per cent lymphocytes 22 per cent thrombocytes 113 000 without structural abnormalities Osmotic resistance of red corpuscles normal Myelogram normal. Spleen puncture see Table 32

Course The lipæmia remained unchanged even on a fat poor diet while a high carbohydrate intake plus insulin also had no effect No increase of fæcal fat Discharged from hospital at the end of 3 months with the splenomegaly, lipæmia and slight albuminuria unchanged

In this case a man of 28 had a distinctly enlarged spleen, lipæmia (*fatty acids between 1 600 and 3 600 mg per cent*), *cholesterinæmia* (324 mg per cent) persistent but slight albuminuria and no signs of renal damage All other investigations proved negative, so that a lipoid nephrosis can be excluded Spleen puncture revealed an almost normal splenogram with a few mature myelocytes (1 per cent) and an occasional erythroblast (0 1 per cent) *No lipophages were found even after very painstaking search of spleen films* This is strong evidence against any type of xanthomatosis Perhaps we are dealing with a previously unknown disturbance of fat metabolism *in which the splenomegaly was purely fortuitous*

TABLE 32

Hæmogram		Pulp cells	0 2
Hæmoglobin (%)	110	Erythroblasts	0 1
Red corpuscles (in millions)	5 040	Myelocytes immature	0 1
Thrombocytes	22½ ‰	Myelocytes half mature	0 2
	113 000	Myelocytes mature	0 1
Leucocytes	6 900	Metamyelocytes	0 6
Metamyelocytes	½	Total myelocytes	1 0
Neutrophiles staff	13½	Neutrophiles staff	6 2
Neutrophiles segmented	59½	Neutrophiles segmented	24 5
Eosinophiles	1½	Eosinophiles	1 5
Basophiles	½	Basophiles	0 3
Monocytes	2½	Monocytes	2 4
Lymphocytes	22	Lymphoblasts	0 2
		Lymphocytes young	6 6
Splenogram		Lymphocytes old	55 6
Plasmacyt retic cells	1 1	Lymphocytes total	62 4
Tissue mast cells	+	Plasma cells	0·3

Harslof (1948) has described a form of familial hepato splenomegaly accompanied by hyperlipæmia colitis hæmorrhagic diathesis (thrombocytopenia) and endocrine disturbance Tannhauser (1937) had previously recorded this disease There is enormous increase of neutral fat in the serum and a moderate increase of cholesterol and lipoids In spite of the absence of enlargement of the liver colitis and endocrine disturbances it is possible that our case is of the same type

SPLENOMEGALY WITH LYMPHOCYTOSIS (POSSIBLY OF CENTRAL ORIGIN)

We have already described a case (p 91) in which a patient with a pituitary tumour had splenomegaly and 18 000 lymphocytes per c mm We regarded this probably as a lymphatic reaction of central origin because an infective or leukæmic origin could be excluded Perhaps there was a temporary decrease of the adrenotropic hormone of the pituitary because other signs of pituitary deficiency were also present This would be in conformity with the experimental findings of Dougherty and White on the regulation of lymphocytopoiesis by this hormone (see our chapter on the lymphocytes p 42)

reticulocytes 1 6 per cent leucocytes 6 900 metamyelocytes ½ per cent staffs 13½ per cent segmented 59½ per cent eosinophiles 1½ per cent basophiles ½ per cent monocytes 2¼ per cent lymphocytes 22 per cent thrombocytes 113 000 without structural abnormalities *Osmotic resistance of red corpuscles* normal *Myelogram*, normal *Spleen puncture* see Table 32

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Neutrophiles segmented	59½	Neutrophiles segmented	24 5
Eosinophiles	1½	Eosinophiles	1 5
Basophiles	½	Basophiles	0 3
Monocytes	2½	Monocytes	2 4
Lymphocytes	22	Lymphoblasts	0 2
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Splenogram		Lymphocytes old	55 6
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another if there is not too much admixture of blood which can be avoided by very short aspiration At least 1 000 cells should be examined

For diagnostic purposes it is not usually necessary to determine an exact splenogram careful examination of films will give a sufficiently accurate idea of the distribution of the cells and of the presence of abnormal ones Of course serial splenograms in leukæmias especially if myelograms are available are specially instructive If there is distinct increase of lymphocytes a full count should be done because it may be the only way to show that there is a aleukæmic form of lymphatic leukæmia restricted to the spleen

Mitosis These are strikingly rare in lymphocytes and we have here given the first quantitative estimations ever published Thus a spleen removed by operation in a case with lymphatic hyperplasia showed only 2 mitoses of lymphoblasts in 50 000 lymphocytes The mitotic index of lymphocytes is thus 50 times less than that of the granulocytes *We have therefore drawn the inference that the life span of lymphocytes as compared with that of granulocytes must be fairly long*

In *lymphatic leukæmia* with many lymphoblasts the mitoses may be increased to 15 or 30 times the normal but this does not occur in all cases In young lymphocytes mitoses are so rare that we have only seen two The idea of amitotic division is not accepted and the evidence against its occurrence is presented in some detail

In *Section II* the characters of spleen puncture fluid in various types of splenomegaly are described

In *cirrhosis of the liver* and in spleno portal thrombosis the splenogram is practically normal even although the spleen may be greatly enlarged It is likely however to be unusually bloody In cirrhosis we did find increase of plasmacytoid reticulum cells in both the spleen and the marrow In cases of inflammatory cirrhosis there were signs of extramedullary blood formation in the spleen even a few megakaryocytes being found

A fairly normal picture was found in *hæmolytic anæmias* but as a result of the increased blood destruction hæmosiderin-containing macrophages were plentiful while some erythroblasts were found during periods of exacerbation We never observed any signs of phagocytosis of red corpuscles

In *pernicious anæmia* megaloblasts are plentiful in the spleen an indication that in the absence of hæmopoietic factor even the reticulum cells of this organ give rise to these abnormal elements Probably the megaloblasts found in the peripheral blood are derived from such extramedullary foci

We draw a distinction between an *acute inflammatory type* of spleen puncture and a *chronic inflammatory* one In the former the lymphocytes are reduced while neutrophiles including staff cells

SUMMARY

Our hæmatological and differential diagnostic descriptions are based on study of over 300 spleen punctures

With proper technique spleen puncture can now be considered as being devoid of danger, if only enlarged spleens are submitted to the procedure and if it is never carried out in the presence of a hæmorrhagic diathesis an acute septic spleen or on comatose patients We would emphasise as do Nagy and Weil, that spleen puncture must only be performed during full inspiration

The *technique* had been modified so that penetration of the spleen is not more than 1¼–2 cm This has been done by using the needle with which the local anæsthetic is given to determine the depth at which the spleen lies both because there is pain when it reaches the peritoneum and also because its end can eventually be felt rubbing against the capsule of the organ Then the guard on the spleen puncture needle is fixed 1½ cm deeper than this Using this tech nique we have never experienced any complication

The *material obtained* is usually 1–3 drops of rather bloody fluid in the needle and a few fragments of tissue It contains 60–80 per cent of lymphocytes together with some granulocytes and reticulo endothelial cells which are mainly derived from the red pulp *i e* the sinuses which are of course not connected directly with the general circulation We would call special attention to the element we have named pulp cell which is probably identical with the sinus endothelium of the histologists As it is not found in gland or marrow punctures it can be regarded as characteristic of the spleen Other important elements are the typical plasmacytoid reticulum cells (identical with those of the marrow) and large lymphatic plasma cells (of the same type as those seen in gland puncture) We regard these two forms as having different origins Thus there are distinct differences of structure while in cirrhosis of the liver there is increase of plasmacytoid reticulum cells in the spleen and marrow without any increase of the lymphatic type And conversely in epidemic hepatitis there is increase of lymphatic plasma cells (and many mitoses) in the spleen while marrow puncture fails to reveal an increase of plasmacytoid reticulum cells (Landolt)

We have also called attention to another type of lymphatic element found in spleen puncture viz the large lymphatic reticulum cell This is also found in gland punctures and is probably identical with the germ centre cells seen in sections

Differential counts (splenograms) can be compared with one

In cases of the *Still Chauffard Felty syndrome* there was some times great increase of plasmacytoid reticulum cells and occasionally of eosinophiles Reference is also made to the occurrence of *cyclic agranulocytosis* in this malady

In *malaria* the presence of macrophages containing melanin may allow a diagnosis to be made in cases of persistent splenomegaly even years after infection

The findings of the causative parasites in spleen films is of course very important in *kala a ar*

We have here given the first description of *tuberculous epithelioid cells* in spleen puncture films This finding is specially important in those cases of chronic splenic tuberculosis which can easily be confused with Hodgkin s disease and other causes of splenic enlargement

In 5 out of 7 cases of *brucellosis* we found large epithelioid cells in spleen films This is the first description of these elements in films although they had previously been seen in sections by Loffler and Albertini In one case with negative agglutination tests the diagnosis was made by this method

We have been able to confirm the observations of other workers on the presence of Dorothy Reed cells in spleen films from *Hodgkin s disease* and we have been able to make a diagnosis of the not rarely encountered hepato lienal form by this method when all other means had failed This also applies to cases in which excision or puncture of glands is impossible

Megakaryocytes can be found in spleen films from cases of toxic thrombocytopenia as well as in chronic inflammatory spleno megalies They are rarely absent in chronic myelocytic leucoses

Lymphatic leucoses are characterised by an almost purely lym phocytic picture (92–99 per cent) This permits the diagnosis of aleukæmic lymphadenosis which is confined to the spleen Often but not invariably the lymphatic cells vary greatly in size while lymphoblasts and mitoses may also be prominent

Acute lymphatic leucosis is discussed in the light of an illustrative case and the relationship of this condition to lymphosarcoma is stressed Further the close relation of *lymphadenosis* and *reticulosis* is affirmed while those aleukæmic cases *in which the B S R is* greatly increased are assigned to the latter category

In *chronic myeloid leucoses* the splenogram is mainly myelocytic (20–60 per cent) with 3–35 per cent of erythroblasts If staff and segmented forms exceed the myelocytes the prognosis is relatively good whereas if the latter predominate it can be inferred that the disease is of longer (some years) duration and that neither the response to treatment nor the expectation of life will be as good Usually the myeloblasts form 0 5–1 15 per cent of the cells higher numbers are a bad sign All our cases in which

and myelocytes are increased as are also the pulp cells and macro phages while an occasional erythroblast may be seen

In chronic inflammatory type, in addition to these changes the monocytes plasmacytoid reticulum cells (1–10 per cent) and some times erythroblasts (0 5–10 per cent) are distinctly increased

Hæmopoietic foci in the spleen are thus usual in inflammatory splenomegalies and cells from these foci may enter the blood in sufficient numbers to arouse together with the splenomegalia and leukocytosis a suspicion of early chronic myeloid leukæmia Spleen puncture enables the diagnosis to be settled in leukæmia, the mye locytes are between 20 and 60 per cent whereas in chronic inflam matory splenomegaly they do not rise above 1–5 per cent Even when myeloid metaplasia in *osteosclerosis* and *myelofibrosis* is so intense that myelocytes are numerous in spleen films (23 per cent in one of our cases) the persistence of large numbers of lymphocytes (50–60 per cent) distinguishes the condition clearly from myeloid leukæmia

The *lymphatic reactions* that occur in infectious mononucleosis and in epidemic hepatitis are discussed in some detail

In *glandular fever* at the height of the disease spleen puncture reveals a considerable excess of reticulo endothelial cells some in mitosis with transitions between them and the typical glandular fever cells Clearly then, these elements arise in the spleen whence they pass into the blood This would explain the blood changes that are found in those splenomegalic cases in which there is no enlargement of lymphatic glands Probably other lymphotropic viruses can evoke similar responses

In *Q fever* we found a definite lymphatic reaction with increase of young lymphocytes in the spleen although the absolute number of lymphocytes was normal

In *infective hepatitis* spleen puncture reveals the presence of many of the immature precursors (some being in mitoses) of those lymphatic plasma cells that are found in the blood These are the same as the elements we have called lymphatic plasmoblasts which are found in gland puncture in *rubella* From these findings together with absence of any excess of plasmacytoid reticulum cells in the marrow (in the early stages of infective hepatitis) we infer that the majority of the plasma cells in the blood in this disease are derived from the spleen

An unusual example of a *lymphatic* reaction was seen in a case of pituitary tumour in which the lymphocytes rose to 18 000 and the spleen became enlarged Spleen puncture and the subsequent course of the illness excluded the possibility of lymphatic leukæmia and we incline to the view that the lymphatic reaction was of central origin but a coincidence with a lymphocytosis acuta cannot be excluded

while the number of lymphocytes in the peripheral blood decreases and it is only after prolonged administration (or in unduly susceptible persons) that the granulocytes fall (Moeschlin and Meili 1947) In animal experiments the lymphocytes are far more easily reduced than are the granulocytes except in cats (Moeschlin and Naef 1948 Moeschlin and Bodmer 1949) *The action of urethane in the leukæmias in the therapeutic employed dose is thus a more or less selective damage to the mitotic activity of the dedifferentiated (neoplastic) white cells* The splenogram and the myelogram of the white myeloid series shift towards maturity although more slowly while the erythroblasts increase

In *lymphatic leucoses* the percentage of lymphocytes in the spleen and marrow shows a reduction although not as a rule a very great one

Urethane thus resembles arsenic in its power of inhibiting mitosis

Treatment of a case of chronic myeloid leukæmia with *nitrogen mustard* was also followed by decrease of immature cells Here also there appears to be toxic interference with mitosis in addition to direct destruction of immature cells Similar changes can be observed after *radio active phosphorus* Both these substances have therefore effects very similar to those of X rays

Sections of spleen puncture material in the early stage of a case of *paramyeloblastic leucosis* showed that initially the abnormal cells have a focal arrangement similar to that of sarcomatous metastases Only later is the organ diffusely infiltrated

Spleen punctures from 3 cases of *erythroleukæmia* are described

In a detailed consideration of the *primary malignant reticuloses (reticulo endothelioses)* an attempt based on spleen marrow and gland punctures is made to clarify this condition and to separate it from other diseases to which the name has been improperly applied Special attention is devoted to cases in which only the spleen and glands were infiltrated by the tumour cells which were also present in the blood while the marrow was free from involvement

The characteristic cells are described in detail and the points of distinction between them and other tumour cells are stressed

The disease is regarded as being neoplastic at first being localised but later metastasing so as to present the apparently systematic distribution which has misled pathologists who see only the terminal phase

The relationships of reticulosis lymphadenosis and undifferentiated sarcomas is considered

Special attention has been given to *the changes in the blood proteins* that often occur in such cases and much emphasis is laid on the function of the reticulo endothelium in relation to the formation and transformation of protein substances There is an interesting

the myeloblasts were between 2 5 and 17 per cent, died within 9 months

Comparative examinations of spleen and marrow films show that the cellular picture is more immature in the latter, and we suggest that this may be due to splenogenic (endocrine) inhibition of the myeloid tissue On the other hand, this view seems to be contra dicted by the fact that there were always 3 or 4 times more mitoses in the granulocytes in the marrow than in those in the spleen The mitoses were almost confined to the immature and half mature myelocytes, and it may be that the high incidence of mitoses in the marrow is simply the result of its high content of undifferentiated cells

Spleen punctures allow the effect of *treatment in chronic myeloid leukæmia* to be followed especially when the marrow is also examined *X irradiation is followed by great reduction and matura tion of the myeloid tissue in the spleen whereas typical leukæmic changes persist in the marrow* Similarly the mitotic index in the spleen falls sharply but remains unchanged in the marrow It can therefore be said that the effects of X irradiation in chronic myeloid leukæmia remain localised unirradiated myeloid tissue being unaffected The fact that irradiation of the spleen is followed by almost complete return to normal of the hæmogram (in spite of the persistence of leukæmic changes in the marrow) seems to be clear proof that the abnormal cells so characteristic of the blood picture in chronic myelocytic leucoses, are derived from the spleen (and perhaps the liver) A view first propounded by Rohr

Our view is that chronic myelocytic leukæmia should not at least in the early stages be treated by general irradiation or by irradiation of the bones, because irreparable damage may be done Careful treatment over the spleen will usually suffice

The effects of arsenic have also been investigated by spleen and marrow punctures *In both tissues arsenic produced a decrease in the percentage of granulocytes* (mainly of the myelocytes but also of more mature forms) *together with a relative increase of erythroblasts Arsenic also leads to diminished mitotic activity in both the spleen and the marrow* In one case in which arsenic had been given in small doses for 6 years the number of granulocyte mitoses was reduced to a fortieth

We agree with Forkner that treatment with arsenic is to be pre ferred to radio therapy especially because it increases the expecta tion of life Its effect depends mainly upon toxic inhibition of mitosis as has also been shown by Dustin

In cases of chronic leukæmia treated with *urethane* there was great reduction in the number of mitoses in the abnormal cells in the spleen but the mitotic index of the erythroblasts rises *In normal persons* urethane does not usually inhibit mitotic activity

while the number of lymphocytes in the peripheral blood decreases and it is only after prolonged administration (or in unduly susceptible persons) that the granulocytes fall (Moeschlin and Meili 1947) In animal experiments the lymphocytes are far more easily reduced than are the granulocytes except in cats (Moeschlin and Naef 1948 Moeschlin and Bodmer 1949) *The action of urethane in the leukæmias in the therapeutic employed dose is thus a more or less selective damage to the mitotic activity of the dedifferentiated (neoplastic) white cells* The splenogram and the myelogram of the white myeloid series shift towards maturity although more slowly while the erythroblasts increase

In *lymphatic leucoses* the percentage of lymphocytes in the spleen and marrow shows a reduction although not as a rule a very great one

Urethane thus resembles arsenic in its power of inhibiting mitosis

Treatment of a case of chronic myeloid leukæmia with *nitrogen mustard* was also followed by decrease of immature cells Here also there appears to be toxic interference with mitosis in addition to direct destruction of immature cells Similar changes can be observed *after radio active phosphorus* Both these substances have therefore effects very similar to those of X rays

Sections of spleen puncture material in the early stage of a case of *paramyeloblastic leucosis* showed that initially the abnormal cells have a focal arrangement similar to that of sarcomatous metastases Only later is the organ diffusely infiltrated

Spleen punctures from 3 cases of *erythroleukæmia* are described

In a detailed consideration of the *primary malignant reticuloses (reticulo endothelioses)* an attempt based on spleen marrow and gland punctures is made to clarify this condition and to separate it from other diseases to which the name has been improperly applied Special attention is devoted to cases in which only the spleen and glands were infiltrated by the tumour cells which were also present in the blood while the marrow was free from involvement

The characteristic cells are described in detail and the points of distinction between them and other tumour cells are stressed

The disease is regarded as being neoplastic at first being localised but later metastasing so as to present the apparently systematic distribution which has misled pathologists who see only the terminal phase

The relationships of reticulosis lymphadenosis and undifferentiated sarcomas is considered

Special attention has been given to *the changes in the blood proteins* that often occur in such cases and much emphasis is laid on the function of the reticulo endothelium in relation to the formation and transformation of protein substances There is an interesting

parallelism between reticuloses and plasmocytic myeloma, which also belongs in this group

Our experience shows that changes in the blood proteins do not generally occur in association with those reticuloses in which there is plentiful formation of fibrils, presumably because the protein forming power of the cells is diverted to fibril formation Changes in the proteins (high B S R positive cephalin and cadmium reac tions electrophoretic changes, and abnormal globulins) are more frequently seen in cases in which the fibril formation is absent—presumably the unfixed proteins make their way into the blood

The structure of various kinds of *sarcoma cells* and the possibility of finding *carcinoma cells* (if a mass erroneously supposed to be an enlarged spleen is punctured) is described The occurrence of sar coma and carcinoma cells in groups unlike the isolated cells of lymphosarcoma and reticulosis is regarded as a valuable differential feature

The first observations of lymphosarcoma lymphoblastoma and other sarcomatous cells as seen by *phase contrast microscopy* are here recorded and the method advocated as a valuable aid for the differential diagnosis of tumour cells

Finally some *rare causes of splenic enlargement* are described viz *Gaucher s disease, Niemann Pick disease* and some rather obscure cases of splenomegaly associated with lipæmia

Based on the present examinations the spleen puncture is found to be a harmless and valuable clinical method of examination taken that the proper described technique is used Our observations have led us to regard spleen puncture especially if combined with marrow puncture as being of great clinical value in the hæmatological and diagnostic sense

EPILOGUE

This completes our discussion about spleen puncture We hope that the communicated results have clearly proven the clinical value of this simple method of examination which if properly handled and indicated is quite safe May it take its due place in the clinical examination technique Like all hæmatological and cytological methods spleen puncture requires a certain experience and its use in clinical diagnosis should be in relation to the whole of the clinical picture in each case On this basis spleen puncture is in many cases especially where a simultaneous sternal puncture is made a valuable and safe supplement of the clinical methods of examination of the present day

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